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RiSCC Manual Version 2.0

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Abstract
Regional Sensitivity to Climate Change in Antarctic Terrestrial and Limnetic Ecosystems (RiSCC) is an international research program on Antarctic terrestrial and limnetic organisms and ecosystems. It is sponsored by the Scientific Committee on Antarctic Research (SCAR), a committee of the International Council of Science (ICSU). The RiSCC programme was formally adopted as a research programme under the auspices of the SCAR Working Group on Biology during SCAR XXVI, in Tokyo, Japan, July 2000. A recommendation to approve of this programme was made to the Delegates Meeting of SCAR XXVI. The Delegates agreed to this recommendation. In this manual the agreed methods are being described, which are to be used in RiSCC projects. Methods presented here have been tried in polar regions under extreme conditions, or with organisms obtained from high latitudes, and have been considered appropriate, providing reliable data.

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Regional Sensitivity to Climate Change
In Antarctic Terrestrial and Limnetic Ecosystems

RiSCC

Manual

Version 2.0

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Contents

1 Preface

Regional Sensitivity to Climate Change in Antarctic Terrestrial and Limnetic Ecosystems (RiSCC) is an international research program on Antarctic terrestrial and limnetic organisms and ecosystems. It is sponsored by the Scientific Committee on Antarctic Research (SCAR), a committee of the International Council of Science (ICSU).

The RiSCC programme was formally adopted as a research programme under the auspices of the SCAR Working Group on Biology during SCAR XXVI, in Tokyo, Japan, July 2000. A recommendation to approve of this programme was made to the Delegates Meeting of SCAR XXVI. The Delegates agreed to this recommendation.

In this manual the agreed methods are being described, which are to be used in RiSCC projects. Methods presented here have been tried in polar regions under extreme conditions, or with organisms obtained from high latitudes, and have been considered appropriate, providing reliable data. For each method the name of the person who prepared the protocol is given and at the end of the catalogue the address, telephone, fax and e-mail of every contributor can be found. We welcome an open discussion on the protocols presented here, and any comments, additions, refinements, or feedback from the users will be important.

Methods have been arranged into three levels of complexity: basic, intermediate, and advanced. The terms basic, intermediate and advanced refer to the level of specialist knowledge necessary to execute the type of research described in the particular section.

Basic studies can be executed by all partners. They require a minimum of time and/or equipment. Most of the basic studies are descriptive and are supported by ready-made forms in the annexes of this manual. If properly instructed, non-specialists, and, in some cases, non-scientists can use these forms to collect valuable data.

Intermediate studies require a certain amount of experience with the methods at hand. These studies require more time and expertise.

Advanced studies require specialist knowledge, and in some cases considerable investment in equipment. These studies are best performed as subject of a dedicated field project.

It is recommended that **all** participants in RiSCC follow the basic methodology, whenever they are in the field, as the basic methods provide the proper information to be added to the RiSCC database in a properly formatted way.

The field forms also provide data/information to other RiSCC participants, and will greatly enhance our common goals.

2 The RiSCC program

2.1 SCIENCE PLAN

2.1.1 Introduction

Climate change is the most complex issue confronting humans and all other biota. The present view is that a global mean temperature increase of approximately 2°C, and attendant changes in precipitation regimes, are realistic expectations over the next 50 years[1-3]. Moreover, because large-scale extinctions and movements of species have resulted from major climate changes in the past[4,5], an ability to predict the consequences to biodiversity of climate change is becoming increasingly important[1].

Over the last fifty years air temperatures and moisture regimes on Antarctica and on Sub-Antarctic islands (here after 'the Antarctic') have changed markedly[6,7], and indications are that this trend will continue. In many areas the biota has shown pronounced direct and indirect responses to these changes[6,8-12]. These clear responses, as well as the isolation of Antarctica from other continents, the comparative simplicity of its terrestrial and limnetic ecosystems, the relative ease with each introduced organism can be documented in the region[13], and the presence of many species range boundaries, provide unique and unparalleled opportunities for investigating the likely consequences of climate change for biodiversity[14].

These opportunities result both from the pronounced rates of change in the region, as well as marked environmental gradients in terrestrial and limnetic ecosystems. Antarctic communities range from polar deserts and permanently frozen lakes to lush, eutrophic grasslands and nutrient enriched ponds. Such latitudinal, and in many cases altitudinal, gradients provide useful analogues for future change.

Regional differences in the nature of the changes add a further, useful layer of complexity. Strong seasonal differences in the rate of temperature change have been observed in the Antarctic Peninsula region, with autumn and winter temperatures having increased substantially more than those in the spring and summer[15]. Precipitation rates in the Peninsula area are also predicted to increase whereas they have been declining dramatically on several Sub-Antarctic islands.

In consequence, the region provides a suite of scenarios useful for investigating the range of climate change effects on terrestrial and limnetic biotas. The overall rationale for the program is to investigate these scenarios with the goals of

1) Understanding the likely response of Antarctic biotas to changing climates;
2) Contributing to the development of broadly applicable theory concerning interactions between climate change, indigenous and introduced species, and ecosystem functioning.
3)

2.1.2 Aims

To understand the interactions between biodiversity, functioning and climate of Antarctic terrestrial and limnetic ecosystems, and to predict regional sensitivity to the impacts of climate change by

1) identifying and quantifying differences in environments and biodiversity within and between ecosystems;
2) understanding the potential for ecosystem processes to respond to changes in climate;
3) partitioning the effects of climate change among the key components of the ecosystems;
4) using new and existing data to provide a synthesis of the likely effects of climate change on Antarctic terrestrial and limnetic ecosystems to contribute to their management and conservation;
5) orienting the research to achieve links with other international programmes seeking to understand the implications of global changes.

2.1.3 Rationale

Species richness forms the major surrogate measure for biodiversity. Climate change will have a significant effect on species richness of ecosystems by altering energy, water availability and nutrients, the major determinants of diversity. The way in which such a change will occur is fundamentally a response of individuals, populations and communities to abiotic and biotic variables. To predict the form these changes are likely to take, a synthetic understanding of the nature of the relationships between performance, plasticity and genetic variation will be a critical step. These relationships will determine the abundance of species, their distributions and, ultimately, the species richness of a given site.

One way of examining the predicted consequences of climate change is to investigate latitudinal and altitudinal gradients as an analogy for future climate change. An Antarctic Environmental Gradient (AEG) exists that covers approximately 30 degrees of latitude and includes a range of macro-climatic zones from cool temperate islands to the frigid and arid continent. A thorough understanding of the influence of abiotic variables on individual and population performance and consequently the relationship between species abundance and species richness along the AEG will provide a robust model for examining the likely effects of climate change.

The Antarctic Environmental Gradient offers a unique opportunity to provide a co-ordinated investigation of the effects of global climate change on natural ecosystems. This opportunity arises because of the coincidence of several important features.

1) Climate changes are predicted to be greatest at high latitudes and have been identified in polar regions;
2) Considerable changes in species richness, abundance and functions, such as performance and productivity, exist along the AEG. There are gradients in complexity of communities along the AEG that offer the opportunity to construct models initially for relatively simple systems and then to extend them to higher levels of complexity;
3) Major groups of organisms reach their distributional limits within the AEG, and the variety of taxa offers opportunities for exploring adaptation to particular climatic factors;
4) The Antarctic is isolated from all other continents. Therefore Antarctic terrestrial and limnetic environments have, until recently been remote from direct human influence. Consequently, many communities remain pristine.

This program will contribute to the management and conservation of Antarctic communities, and provide insight into the effects of climate change on other ecosystems.

1. What factors influence species richness and functional group diversity along the AEG, and how will changes in species richness and functional group diversity affect ecosystem structure and functioning?

2. How do organisms and communities respond to abiotic variables along the AEG and how will climate change influence these responses?

3. How do phenotypic plasticity and genetic variation and their interaction differ in key groups within and between sites along the AEG?

2.1.4 Key Scientific Questions

1. What factors influence species richness and functional group diversity along the AEG, and how will changes in species richness and functional group diversity affect ecosystem structure and functioning?

In the Antarctic we have the opportunity to develop a substantial picture of biodiversity in one of the Earth's largest biomes. Along the AEG, the main factors influencing species richness and functional group diversity differ substantially. Historical factors, such as palaeoclimate and plate tectonics, provide the baseline on which changes in temperature, water availability, nutrients and human activity act to influence this diversity. The significance of these latter factors, and the impact of climate change on them, form the major topic of this question, which will be addressed by investigations at a variety of sites along the AEG.

The relationship between species richness and functional group diversity may be important in determining a system's resilience and resistance to change. The gradient in species richness from relatively species poor and trophically simple high Antarctic to the more species rich and complex Sub-Antarctic systems provides a useful tool for assessing this relationship. To detect such changes a robust baseline data set (existing and novel), both for the environmental factors and the organisms, is essential. This will require the designation of a series of sites along the AEG that will provide a means to address the following questions:

1.1. What are the differences in the species richness and/or functional diversity at each of the identified sites along the gradient?

1.2. What are the differences in the relationships between this richness/diversity and environmental factors, isolation and history along the AEG.

1.3. When do biotic interactions become important in regulating species richness/functional group diversity?

1.4. To what extent do introduced species alter species richness and functional diversity?

1.5. Can change in diversity within sites be detected under a climate change scenario by

1.5.1. monitoring undisturbed sites along the AEG,

1.5.2. observing colonization and establishment along the AEG,

1.5.3. i*n situ* and *ex situ* manipulations of organisms/communities at various hierarchical levels.

2. How do organisms and communities respond to abiotic variables along the AEG and how will climate change influence these responses?

Abiotic factors such as irradiance, temperature, water availability, nutrient concentrations, and CO_2- and O_2-levels are important factors controlling performance of Antarctic terrestrial and limnetic organisms. These factors vary both within and between seasons and sites, and this variation encompasses freeze/thaw and wet/dry cycles. The optimum levels of these factors and the thresholds at which the organisms are still able to function should be determined. Changes in the relationship between abiotic factors and performance will profoundly influence life histories and productivity of Antarctic organisms. These relationships in turn will influence the distribution and abundance of organisms and community structure. In particular there is a close link between terrestrial and limnetic ecosystems such that changes in the former have a profound effect on the latter.

To provide answers to this major research question, the following issues must be addressed using selected species:

2.1. Which abiotic factors control productivity and reproduction and how do they vary both temporally and spatially within and between sites along the AEG?

2.2. How will climate change alter the relationship between physiological and life history traits?

2.3. How will climate change-induced variations in terrestrial systems affect limnetic ystems?

2.4. How will ecosystem functioning alter with climate change along the AEG?

3. How do phenotypic plasticity and genetic variation and their interaction differ in key groups within and between sites along the AEG?

The combination of different degrees of geographical isolation, time since colonisation, species biology and climatic gradients will lead to variation in response to climate change between species and populations. Through phenotypic plasticity and/or genetic variation, populations will have different capacities to respond to the effects of climate change. These differences in response will lead to changes in community structure and dynamics.

3.1. What is the extent of phenotypic plasticity and/or genetic variation within populations of key groups at sites along the AEG?

3.2. How will phenotypic plasticity and/or genetic variation affect the response of key groups to climate change?

2.1.5 References

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2.1.6 Links to other programs

Programs sponsored by SCAR:

ANTIME (Late Quaternary Sedimentary Record of the Antarctic Ice Margin Evolution)

ANTIME examines climate, environmental and ice sheet history over the last glacial cycle (130,000 years) and in detail, the last 10,000 years. Changes in the Antarctic climate, studied within ANTIME, will provide information for the historical background of species distributions.

ITASE (International Trans-Antarctic Scientific Expedition)

ITASE focuses on high resolution climate and environmental history covering the last 200 - 500 years. The output of this program is directly relevant as climatic background information on species distributions.

EASIZ (Ecology of the Antarctic Sea Ice Zone)

Components of the EASIZ program such as the seasonal, inter-annual and long-term changes in coastal and pack-ice zone ecosystems, their associated links to climate fluctuations, and the ecosystems controls on biogeochemical fluxes form a natural connection with the terrestrial and limnetic studies of RiSCC in the coastal zone.

EVOLANTA (Evolution in Antarctica)

Evolanta will examine genetic variation in terrestrial and limnetic organisms. It will provide information on the evolutionary history of the organisms which will also be examined in RiSCC.

Non-SCAR programs:

PAGES (Past Global Changes) (IGBP)

The SCAR-programs ANTIME and ITASE are co-sponsored by the IGBP core project PAGES, which co-ordinates on a world-wide scale programs on the study of those aspects of past global change that contribute crucially to our understanding of Earth system functioning on human timescales and to our capacity to improve the quality of any asessment of future climate changes and their impacts. As such, RiSCC and PAGES science plans have congruent aims in the Antarctic.

GCTE (Global Change and Terrestrial Ecosystems) (IGBP)

GCTE aims to predict the effects of changes in climate, (and atmospheric composition and land use) on terrestrial ecosystems. The research questions of RiSCC fit three of the four Foci of GCTE:
1. Ecosystem Physiology
2. Change in Ecosystem Structure
3. Global Change and Ecosystem Complexity (Biodiversity)

Within GCTE there is a growing emphasis on the regional aspects of global change. To this effect, 14 Terrestrial Transects have been established worldwide, ranging from the Arctic (Alaskan Latitudinal Gradient, the Siberian Far East Transect and the Europe transect) to the humid tropics (SE Asian Transect, the Amazon). Discussion between the GCTE Executive Officer and the Steering Committee of RiSCC has started to add the RiSCC AEG to this list as the 15th Terrestrial Transect.

ITEX (International Tundra Experiment) (MAB)

The purpose of ITEX is to monitor the performance of plant species and communities in undisturbed habitats with and without environmental manipulations throughout the circumpolar Arctic and in some alpine areas. The basic experiment is a temperature enhancement manipulation, where the field mean surface temperature is increased by 2-3 degrees C to simulate the climate at the middle of the next century according to the forecast from the GCMs (General Circulation Models). ITEX is therefore the natural Arctic counterpart of RiSCC and plans for collaborative research are being discussed.

I-LTER (International Long-Term Ecological Research) (NSF-USA)

The LTER Network is a collaborative effort promoting synthesis and comparative research across sites and ecosystems and among related research programs. The Palmer LTER-site and the McMurdo Dry Valleys LTER-site have been incorporated in the RiSCC AEG.

DIVERSITAS (International Programme of Biodiversity Science)
(IUBS, SCOPE, UNESCO, ICSU, IGBP-GCTE, IUMS)

The preceding SCAR program on Terrestrial Ecological Research (BIOTAS) was ncorporated in DIVERSITAS (Programme Element 4: Monitoring of Biodiversity). Negotiations between the Steering Committees of Programme Elements 1 (The Effect of Biodiversity on Ecosystem Functioning) and Programme Element 4 and the RiSCC Steering Committee have commenced to have RiSCC affiliated to these Programme Elements.

LGP (Latitudinal Gradient Project)

LGP is a multinational, interdisciplinary initiative aimed at increasing the understanding of the coastal marine, freshwater and terrestrial ecosystems that exist along the Victoria Land coastline in the Ross Sea region, and describing potential environmental variability that may occur in the future. Five sites along the Victoria Land coast have been chosen for study over a 10-year period with two years of research at each site. These sites have been incorporated into the RiSCC AEG as the key questions of LGP and RiSCC are comparable. For more information contact Shulamit Gordon (LGP Project Manager) at s.gordon@antarcticanz.govt.nz or visit www.antarcticanz.govt.nz

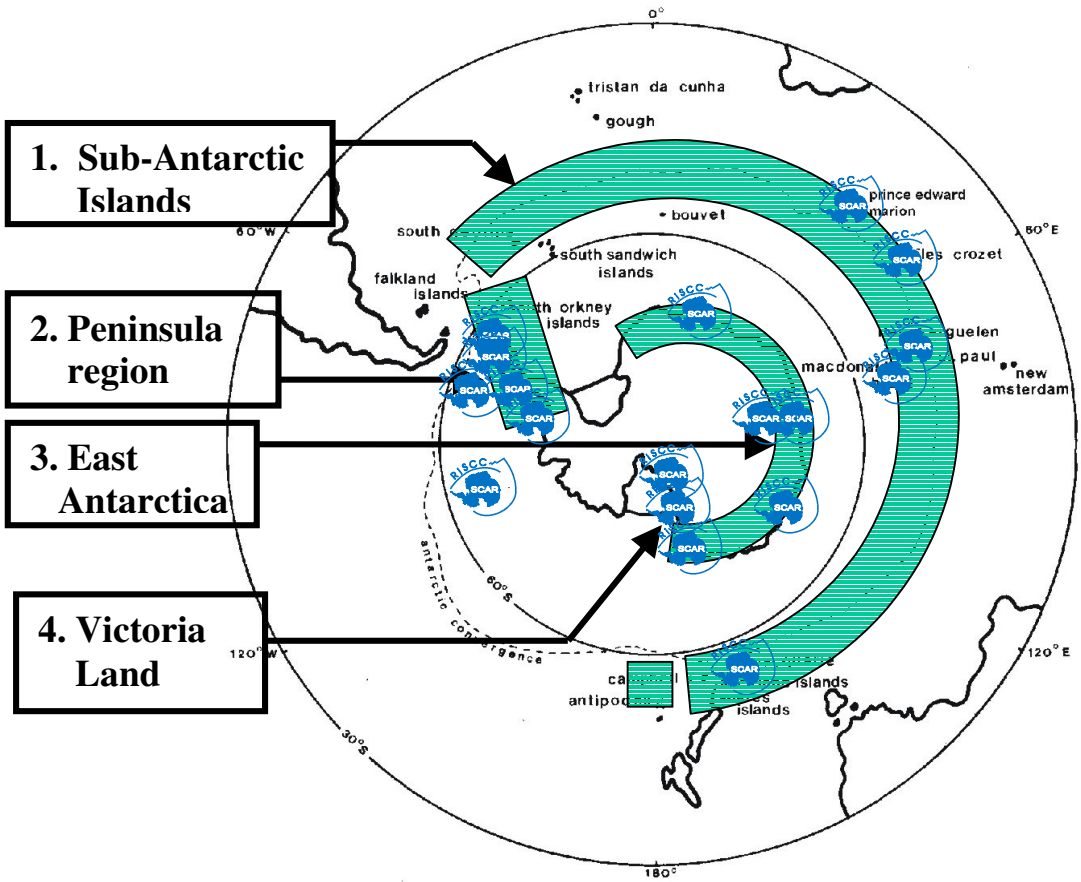
2.2 IMPLEMENTATION PLAN

Recognizing that there is a wealth of information available with which to address the key questions being posed by RISCC a major part of this research program will be devoted to making these data available in a form that is suitable for analysis. These data will constitute the baseline on which the RISCC science program will be constructed.

Furthermore, new information of two kinds will be added. First, additional data on species richness and functional diversity will be collected to improve the biodiversity database. Second, novel experimental approaches will be adopted across the AEG to allow effective examination of the responses of this biodiversity to climate change. Bearing these major approaches in mind, the RISCC program will identify a set of sites and organisms that will constitute the foundation of its core program, and a suite of approaches that will enable interested collaborators to widen the applicability of the research.

For the program to be successful the techniques and approaches adopted must be cross-validated to ensure comparability across sites. At the same time, the RISCC program recognises that different research groups may adopt somewhat different techniques, which, so long as they are cross-validated, remain useful and important. While the strength of the RISCC science plan stems from addressing questions across the Antarctic Environmental Gradient, the diversity of habitats and climates represented by this gradient necessitate a flexible approach.

2.2.1 The Antarctic Environmental Gradient (AEG)



The AEG core sites are those for which a reasonably well developed set of abiotic and biodiversity data exist. These sites are as follows:

Region	Site	Lat	Long
Sub-Antarctic	Marion Island	47	38E
	Possession Island	46	52E
	Macquarie Island	54	159E
	Kerguelen Islands	49	70E
	Heard Island	53	73E
Peninsula	Signy Island	60	45W
	King George Island	62	58W
	Juan Carlos I	62	60W
	Hope Bay/ Esperanza	63	57W
	Primavera	64	61W
	Palmer	64	63W
	Rothera Point	67	68W
	Mars Oasis	71	68W
Coastal Continental Antarctic	Casey	66	110E
	Vestfold Hills	68	78E
	Larseman Hills	69	76E
	Syowa	69	39E
Victoria Land	Dry Valleys	77	163E
	Cape Hallett (LGP)	72	170E
	Terra Nova Bay (LGP)	74	164E
	Granite Harbour (LGP)	76	162E
	Darwin Glacier (LGP)	79	159E
	Beardmore Glacier (LGP)	84	171E

2.2.2 The core organisms

Higher taxon	Species
Macroscopic algae	*Prasiola crispa*
Lichens	*Usnea* spp.
	Umbilicaria spp.
Mosses	*Ceratodon pupureus*
	Polytrichastrum alpinum
	Sanionia spp.
	Andreaea spp.
	Bryum spp.
Vascular plants	*Acaena magellanica*
	Agrostis magellanica
	Azorella spp.
	Blechnum penna-marina
	Colobanthus quitensis
	Deschampsia antarctica
	Poa spp.
	Pringlea antiscorbutica
Invertebrates	*Alaskozetes antarcticus*
	Halozetes belgicae
	Cryptopygus antarcticus
	Belgica antarctica
	Parochlus steinenii
	Telmatogeton spp.
	Nematodes

2.2.3 Addressing the Key Scientific Questions

The Key Scientific Questions will be addressed using data from core sites within the AEG, as well as data from non-core areas. The latter information will be particularly useful for addressing Key Scientific Question 1.

Key Scientific Question 1: What factors influence species richness and functional group diversity along the AEG, and how will changes in species richness and functional group diversity affect ecosystem structure and functioning?

This question will be addressed by creating a biodiversity database and cross-linking it to abiotic information. These data will be geo-referenced at the finest resolution available, and will be obtained both for the core sites and for the wider area. The extent to which this Key Scientific Question can be effectively addressed depends critically on the careful design and subsequent population of the biodiversity database, the creation of appropriate metadata records and the development of effective cross-links to the abiotic information, both existing and newly collected. The importance of this task for realising the goals of Key Scientific Question 1 cannot be overemphasized. Hence, substantial work will go into this task. The database will serve as a tool that can be used to address this key scientific question, as well as other questions such as those associated with State of the Environment Reports.

The biodiversity database will consist of a master taxonomy list and two subordinate lists that will provide information on either observations (those records with no specimens accessioned in collections) or collections (specimens accessioned). These lists will each contain information that will be cross-referenced to two types of metadata records. The first will be a single metadata record referring to the database as a whole. The second kind will be created for each activity associated with RISCC research.

This database will allow Q1.1 and Q1.4, and to some extent issues raised under Q1.5 of this key scientific question to be addressed. For Q1.2, the biodiversity database will have to be cross-linked to abiotic information. This information will be available at a variety of resolutions for each site. Such abiotic information will also be collected at an appropriate resolution *de novo* at the core scientific sites.

Q1.3 will be addressed by encouraging the appropriate kinds of investigations required to identify biotic interactions at each of the AEG core sites.

In addition to analytical database work, Q1.5 will be investigated by establishing permanent plots in areas of high and low human activity at AEG core sites to detect colonization, establishment and change. Furthermore, *in situ* and *ex situ* manipulations of abiotic variables will be undertaken using methods appropriate to the sites and/or organisms under investigation.

Key Scientific Question 2: How do organisms and communities respond to abiotic variables along the AEG and how will climate change influence these responses?

To address Q2.1, spatial and temporal variation in abiotic variables must first be investigated. This will require collation of data that are comparable at each of several scales (e.g. satellite, macroclimate, microclimate) and that can be linked to the biodiversity database. It will also necessitate the establishment or continuation of climate logging at the core sites using comparable (or cross-validated) instrumentation. It is recognised that within each of the major regions constituting the AEG, the biological limiting factors are likely to differ. Thus both shared (e.g. temperature) and unique (e.g. ice melt, precipitation, and both) abiotic parameters will be measured. Measurements compatible with protocols of the International Permafrost Association (IPA) will be undertaken at relevant

Core group	Character suite	Characters
Microorganisms	Physiological	Photosynthetic, chemosynthetic and respiration rates
		Uptake, assimilation and allocation
		Temperature and desiccation tolerance
		Growth rates
Macroscopic algae	Physiological	Photosynthetic and respiration rates
		Water balance
		Growth rates
Mosses & Lichens	Morphology	Size
	Physiological	Photosynthetic and respiration rates
		Water balance
	Reproductive	Mode; spore counts; vegetative characteristics
Vascular plants	Morphology	Size
	Physiological	Photosynthetic and respiration rates
		Water use efficiency
	Reproductive	Inflorescence number; seed weight, seed number
Invertebrates	Morphology	Size
	Physiological	Respiration rate
		Thermal tolerance
	Reproductive	Egg size and number

Using a variety of analytical techniques, relationships between the physiological and reproductive characteristics will be sought, and in so doing Q2.2 will be addressed.

A third step is to investigate community responses (eg changes in biomass and nutrient cycling) to abiotic variables. Resolving community responses is especially relevant to limnetic systems.

To identify the ways, in which the links between terrestrial and limnetic systems will be altered by climate change (Q2.3), element (C, N, S, P) flux through these systems will be investigated. This may be done using stable isotope signatures and organic compounds that can be used as biomarkers.

<u>Key Scientific Question 3</u>: How do phenotypic plasticity and genetic variation and their interaction differ in key groups within and between sites along the AEG?

This Key Scientific Question will be addressed by investigating the core organisms at the AEG core sites. In addition, the RISCC science program will encourage work on a broader array of organisms at a variety of sites to encompass the variation in responses likely to be shown by Antarctic biota. For example, lichens are a group that will be studied at some sites, but not at others, and this applies to a variety of other groups across the AEG.

Genetic variation and phenotypic plasticity will be investigated in core organisms at core sites in the following way. For genetic variation, research groups will be free to adopt those measures of variation that are most appropriate to the biota being studied. A prerequisite of this work is that all appropriate sequence information be entered into Genbank and cross-referenced on a RISCC activity metadata record. For the work on phenotypic plasticity, morphological, physiological and reproductive characteristics will be investigated at a minimum of three acclimation and three experimental temperatures (in each case 0, 5 and 10°C). In each of the higher groups the specific characteristics that will be examined are as follows:

Higher group	Character suite	Characters
Macroscopic algae	Morphological	Form variation
	Physiological	Photosynthetic efficiency; water balance
Lichens	Morphological	Size and growth form
	Physiological	Chlorophyll content, Photosynthetic efficiency; water balance
	Reproductive	Reproductive characteristics
Mosses	Morphological	Size, shoot density
	Physiological	Photosynthetic efficiency; water balance
	Reproductive	Mode; spore counts; vegetative characteristics
Vascular plants	Morphological	Specific leaf area; stomatal density; height of plant; root system
	Physiological	Photosynthetic efficiency; water use efficiency
	Reproductive	Inflorescence number; seed weight
Invertebrates	Morphological	Body size; Fluctuating asymmetry
	Physiological	Respiration rate; Thermal tolerance
	Reproductive	Egg size and number

2.2.4 Timetable

Workshops will form an important component of the RiSCC program. Workshops will include those devoted to cross-validation of techniques, database design, population and analysis, and cross-linking of existing data from other sources to the RiSCC biodiversity database. These workshops will be among the major milestones for the RISCC program which can be outlined provisionally as follows:

2000: Effective start of the RiSCC program following approval by SCAR
2000 - 1: Development of details of suggested protocols via e-mail
2001: Workshop on database design and loading, and analysis, presentation of the suggested protocols in conjunction with the VIIIth SCAR Biology Symposium.
2002: Workshop on syntheses of existing data and cross-validation workshop: ecophysiological and AWS measurements.
2002: RiSCC field work formally commences
2003: Workshop outcome of first field season
2005: Major review of RiSCC program
2006: Gap filling AEG expedition
2009: Formal end of RiSCC program
2011: RiSCC Final Symposium

2.2.5 Scientific Programme Group

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2.2.6 Co-ordinators responsible for research protocols

For each of the Key Scientific Questions a research coordinator in both the terrestrial and limetic areas was identified (see below) at the second RiSCC workshop in the Kruger National Park, South Africa. These coordinators are responsible for the research protocols:

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2.2.7 Custodians of the major taxonomy lists of Research Focus 1

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3 Planning field investigations and establishing field sites.

(Cynan Ellis-Evans; modified from Ron Lewis Smith's contribution to the BIOTAS manual)

3.1 PLANNING FIELD INVESTIGATIONS

Any biological or environmental study to be undertaken at a field site in the Antarctic or sub-Antarctic needs to address a suite of formal and practical questions. The following questionnaire is suggested as a guide to aid principal investigators and research managers when planning their research programme. The key questions are by no means complete.

(a) What are the objectives of the investigation?

(b) Will it provide a major contribution to:
- global science?
- Antarctic science?
- regional science?
- local science?

(c) Will it provide new, fundamental information for:
- the Antarctic?
- the region?
- the site?

(d) Are you aware of similar or related studies, where these were undertaken and by whom? If so, are you familiar with the relevant published results? Will your studies benefit by consulting or collaborating with these workers? Will your investigation repeat studies already undertaken in:
- the Antarctic?
- the region?
- the site?

If so, is the proposed study really necessary?

(e) Will your research programme be adequately funded, facilitated and competently staffed so as to yield valid results which will make a worthwhile contribution to science and justify the cost and effort? If there is doubt about this, you should consider carefully what the benefits, if any, are going to be.

(f) If there is a choice of localities where the study could be undertaken, what criteria should be considered for its selection?
- logistics?
- facilities?
- access to field site?
- Antarctic Treaty and/or national protocol restrictions or requirements?

(g) Once a locality has been chosen, what criteria should be considered for site selection?
- access?
- distance from operational base (e.g. station, ship, aircraft)?
- extent of ecosystem, community, physical feature?
- representativeness for purpose of study?
- waste disposal measures?

(h) Are you conversant with the Antarctic Treaty Recommendations (referred to as Antarctic Treaty Consultative Meeting or ATCM Recommendations) relating to research in the Antarctic? If not, do you know where to locate a copy of the most recent edition of your national Handbook of the Antarctic Treaty, or relevant issues of Scientific Committee for Antarctic Research (SCAR) Reports and Bulletins?

(i) Are you conversant with national legislation relating to working on sub-Antarctic islands? If not, do you know where to obtain this information?

(j) Will your proposed studies be in a Site of Special Scientific Interest? If so, have you consulted your national operator responsible for the administration of Antarctic expeditions for approval to work in the Site? [See *ATCM Recommendation VIII-3, sections 3-4*].

(k) Will your proposed studies be close to or, for a compelling scientific purpose, within a Specially Protected Area? If close to a SPA, is your presence or research likely to influence any aspect for which the SPA has been

designated? If within a SPA, has a permit been issued in accordance *with ATCM Recommendation III-8, Article VIII, sections 2-4*?

(l) Following an Environmental Assessment of your proposed field research programme, as required by the Antarctic Treaty for all field projects, will an Initial Impact Assessment or Comprehensive Environmental Assessment have to be prepared? [See *General Provisions for Environmental Protection in ATCM VI-4, VII-1, VIII-13, IX-5, IX Report, XV-1, XV-2, XV Report sections 40-67* together with *Comprehensive Measures for the Protection of the Antarctic Environment and Dependent and Associated Ecosystems*; also *Environmental Impact Assessment in ATCM XII-3, XIV-2 (Guidelines), XIV Report sections 68-70, XV Report sections 96-111*; also *Siting of Stations (facilitation of scientific research) in XIII-6, XIII Report sections 59-60, XIV Report sections 106-112, XV-17, XV Report sections 69, 152-154*].

(m) Will the choice of site and nature of the research programme comply with the Antarctic Treaty Agreed Measures for the Conservation of Antarctic Fauna and Flora*? [ATCM Recommendation I-8, II-2, III-8 Articles I-XIV* and *Annexes A-D, III-9, III-10, IV-18, IV-19, IV-20, VI-9, VI Report, VII-2, VII Report, VIII-2, VIII Report, XIII-14, XIII Report*; *also Recommendation on New Islands contained in VI-11*].

(n) Can the research be undertaken with due regard to the Antarctic Treaty Code of Conduct for Antarctic Expeditions and Station Activities? [*ATCM Recommendations VIII-11 Annex; and subsequent Recommendations on Waste Disposal contained in XII-4, XIII-4, XIV Report sections 71-74, XV-3, XV Report sections 77-88; Use of Radio-Isotopes in VI-5, VI-6, VII Report*].

3.2 SITE SELECTION

3.2.1 Scale of study

The nature of the research programme will determine the dimensions and characteristics of the site required for the study. Such sites should be at least the minimum size in which the study can achieve its objectives. Broadscale surveys typically cover large areas of lakes, ponds or streams (e.g. the RISE project) whereas experimental studies are usually concentrated in small discrete sites. Long-term study sites will need to have a management plan to protect experiments, monitored plots, instrumentation, etc.

Regional (Macro-scale)

Studies being undertaken within a large area, covering one to many square kilometres, e.g.:

(a) Mapping: vegetation, geomorphological or soil features or location of lakes and streams.

(b) Biological survey: phytosociology, ecology, biogeography, inventory (collections of biota), meso- or micro-scale studies in which the size or nature of the site is unimportant and can be undertaken at random throughout the area (e.g. to obtain samples for laboratory experiments)

Ecosystem (Meso-scale)

Studies being undertaken within a site representative of a particular ecological or environmental system, e.g.

(a) Physical feature: hydrology (lake, stream, catchment, etc.), geomorphology (moraine, raised beach, patterned ground, boulder field, outwash plain, sand dunes, etc., which would include permafrost), geology (rock type), topography (rock structure, plateau, ridge, basin, etc.) glaciology (ice recession) and lake type (meromictic, epishelf, etc.)

(b) Biological feature: ecosystem (e.g. specific vegetation or community type, stream type, temporary pools, epishelf lakes), habitat (anoxic sump, benthic mat) or population.

Individual (Micro-scale)

Studies concentrating on individual organisms or groups of organisms within a very restricted area, usually in association with field or laboratory experiments.

3.2.2 Site requirements

It is unwise to select a site without a prior survey, reference to published and unpublished

accounts relating to the site or wider area, and consultation with persons with knowledge of the proposed site. The nature of the research will dictate the site requirements, especially if experimental or monitoring studies are planned. It is usually logistically relevant to address the questions:

(a) Is there a suitable site for the proposed study within convenient distance of, and easily accessible from, the research station/facility from where it will be conducted? If no suitable site lies convenient, should the work be undertaken elsewhere?

(b) Can the site (including its surrounding catchment) support the proposed scale of research programme (numbers of people, frequency of presence, multi-year impact)?

If there is no alternative location for the study, can the programme be adapted to suit the available site and still be scientifically valid?

(c) Numerous features can influence site selection and any one feature may have positive or negative effects depending on the research proposed. A checklist for features worth considering when selecting a site could include:

1. age of ice free area (e.g. deglaciated within past few years, decades ago, centuries ago as evidenced by maturity of surface features and degree of past or present vegetation cover)
2. altitude of site (was it originally marine, access for marine animals, etc.)
3. distance from the sea (seaspray, possible access for equipment by boat)
4. micro-climate (is the site representative of the area or does it have a unique climate?)
5. depth and duration of winter snow, presence of permanent ice/snow.
6. exposure to wind (variable snow cover persistence, sediment resuspension)
7. exposure to solar radiation (shading of part or all of site)
8. presence of local geothermal effects
9. geology (e.g. limestone or igneous outcrops present)
10. nature or texture of substratum (e.g. soil type, grain size, stone/boulder size, etc.)
11. organic deposits in catchment (occurrence, type, depth)
12. depth of permafrost
13. stability of substratum (e.g. subject to freeze-thaw disturbance, solifluction, flooding, wind/particle abrasion, erosion, etc.)
14. biotic perturbation of catchment (influence by birds or seals)
15. biotic nutrient enrichment by vegetation or animals
16. hydrology of site: lake, pool, stream, seepage area, drainage pattern, etc.,
17. hydric state: moisture availability, wet-dry cycles, moisture gradients, etc.
18. homogeneity of site characteristics
19. isolation from nearest ice-free area or lake
20. susceptibility to snow drifting in summer
21. presence of human interference (proximity of buildings, vehicles, Antarctic personnel, tourists, etc.)

When selecting limnetic sites the following features also need to be considered:

22. links to other limnetic sites in the catchment
23. patterns of ice and snow cover on stream or lake
24. stream seasonality (flow periods, inflows and outflows), source (ice or snow).
25. flow rates in streams (highly variable over season?)
26. is the lake an open or closed system or seasonally variable (outflow clues)
27. evidence of seasonal or long term changes in water depth (old shorelines)
28. is the water body likely to be frozen solid in winter (i.e. a pool)
29. is there a seasonal or permanent anoxic sump in the lake?
30. is there meromixis?
31. patterns of particulate loading (glacial flour in spring?)
32. size of water body and of relevant sampling areas in the water body.

The important point to remember when considering limnetic study sites is that these water bodies are sumps for an often much larger terrestrial catchment area and that what happens in the catchment impacts on the water body. For example a limnetic site surrounded by periglacial features or by extensive moss carpets will be influenced over time by trampling damage to these features which may cause land slips, change sub-surface hydrology and inflow chemistry or alter catchment vegetation and flora. Likewise planning a long term benthic sampling study of a small limnetic feature will inevitably result in that feature being destroyed whereas a larger water body may have more resilience.

3.3 SITE ESTABLISHMENT

3.3.1 Site dimensions

The dimensions of the site should be the minimum in which the proposed study or range of studies can be undertaken without overcrowding of activities, experiments, etc. In limnetic studies the site dimensions are usually defined by the size of the water body under investigation. However, where a catchment is being studied some consideration of size is valuable. It is also much easier to manage if it is not too large.

3.3.2 Site demarcation

If the research project is to be completed within one season there may be little need to demarcate the site unless there is a temporary requirement for the duration of the study.

If the study is planned to run for several seasons or the site is to be used for various projects over a number of years, it is important that the boundaries of the site are clearly demarcated. This alerts other personnel not associated with the research programme of the presence of an exclusion or limited access zone which they should not enter without permission of the principal investigator, or as stipulated in the site management plan (see below).

A site map is a useful document and a map can be obtained by plane tabling and compass bearings (or GPS measurements; use differential GPS to get the necessary accuracy).

Marking the site should be done with due consideration to the research and to the environment. Markers should be prominent and placed at intervals of about 50 m around the perimeter of the site, although for large sites the distance apart will have to be much greater. They should not be made from piles of rocks since this involves removing material from the immediate area and disturbing the local substratum. It is preferable to mark the site using posts made of wood (although iron or plastic may be used), about 5 cm x 5 cm, or 5 cm diameter, and at least 1 m high above the ground surface. These must be documented in a stake book.

It is common practise to paint stakes in a bright colour, but paint is not durable and will flake off within a year or two. Marking the tops of stakes with strong adhesive coloured tape is also environmentally unsuitable as it becomes brittle and also flakes off. Consider carefully what effect these flakes or fragments may have on the local environment, and particularly on the studies being undertaken (e.g. they may cause significant anomalies in soil/plant/water chemical analyses).

Plastic labels or caps are perhaps the most durable means of colour-coding posts; these should be fixed using nails or a low temperature resin adhesive. Instructional labels can also be attached to posts; these can be of aluminium marked with metal letter punches. It should not be necessary to link the posts by rope, string, wire or any other fencing material.

3.3.3 Within-site markers

Within the site it may be necessary to delimit experimental plots or sub-sites where specific studies are to be carried out or which will be monitored. These may be marked with short pegs which can still be located during periods of summer snowfall. If it will be necessary to sample these in winter, ensure the markers can be located when there is a metre or two of snow cover. These markers must documented in a stake

book to ensure effective site management.

3.3.4 Within-site routes

Most Antarctic terrestrial systems, as well as lakes and streams and their catchments, are ecologically and environmentally sensitive, and consequently vulnerable to perturbation. Whenever there is concentrated activity within a restricted area, the effects of trampling quickly cause changes; vegetation and periglacial features are easily damaged or destroyed and drainage patterns altered. Such major sites should have clearly designated entry points. The number of entry points should be kept to a minimum, preferably one. It is very important that activity within the site should be restricted to paths for access from the entry point of the site to each of the sub-sites. These paths should be arranged to create the minimum impact on the environment and afford maximum protection within the site. They should be as few and as narrow as possible. If extensive areas of periglacial features, boggy ground or dense stands of moss block access to the water body it may be necessary to install walkways made of timber so as to minimise trampling damage. No vehicular transport (including helicopter landings) should be permitted within the site during the summer months if at all possible. If summer access is necessary to the general area by such transport it should always be by the same route (no overflights if at all possible) and all vehicles left outside the study site catchment, preferably always at the same place. The potential impact of such practices should be part of the environmental assessment at the outset of any project. The US Dry Valley Lakes LTER has produced a very useful document on identifying and addressing scientific impacts.

3.3.5 Field huts and camps within the site

It may be considered necessary to have a field hut or tented accommodation within or adjacent to the site to aid the research, afford shelter during bad weather or provide overnight accommodation. This should be kept to the minimum size that will be practicable for installation and maintenance, while providing the necessary facilities to support the research. Remember that it is now a requirement of the Antarctic Treaty that the erection of even a small hut requires an Environmental Assessment. It is inevitable that such a hut will serve as a focus for depoting and accumulating materials associated with site maintenance and the research programme. It is important that such materials, including all waste, fuel containers, etc. are removed periodically, and that any food rations, fuel, medical kits, etc. are checked regularly and replaced as necessary.

Activities around a hut may influence local hydrology, alter rates of weathering or introduce chemical contaminants to a water body so careful consideration must be given if establishing a site for a hut within a study catchment. Ideally place the hut on bedrock and remote from inflow streams or major seepage areas.

3.3.6 Management of research sites

It is imperative that once a research site has been selected and established, all personnel associated with the parent research station are aware of the:

- location of the site
- restrictions pertaining to the site
- persons involved with the research programme
- programme co-ordinator (in the field and/or in the home country)
- purpose of the research programme

If a site is intended to be used for long-term studies and sustain a range of experimental research, principal investigators may wish to liaise with their national organizations to propose their site as a Site of Special Scientific Interest. This would require the preparation of a management plan, which should be written so as to afford adequate protection to the research programme being conducted there. Proposals should be submitted through the national organization to the SCAR Secretariat (Scott Polar Research Institute, Lensfield Road, Cambridge, U.K.) for consideration by the SCAR Group of Specialists on Environmental Affairs and Conservation. It will then be passed to SCAR for approval and finally to the next Antarctic Treaty Consultative Meeting for ratification.

Each significant research site should have its own

specific management plan or Code of Conduct prepared and regularly reviewed by the principal investigators, in collaboration with the station leader. The information it contains should be in the form of an easily understood "Use of Site" protocol.

It is the responsibility of the national operator and/or principal investigator to provide the station leader with this information, and to ensure that all station personnel are aware of the nature and location of the research programme, and comply with any regulations requested. This is best done by having a set of brief, clear and simple instructions, with a map of the site, posted on the main station notice board.

Principal investigators must also ensure that station leaders take precautions against possible interference at stations that entertain tourists and parties of non-scientific visitors. Remember that tourists, and many non-scientific station personnel, are very interested in the scientific activity at a station, being one of the attractions of visiting the Antarctic. While the station leader is responsible for tourist activity at and near the station, and appreciating that tour visits can be very disruptive to a research programme, it is none the less good public relations to treat such interruptions positively. Such visitors are always inquisitive but very appreciative of explanations and often take a genuine interest in the science being undertaken - often at their expense as taxpayers!Site maintenance

Sites designated for long-term studies must be maintained in good order; otherwise the science may suffer. They must be inspected at regular intervals to check that markers and any other site artifacts are in place. This is particularly important at the end of winter and after storms.

3.3.7 Termination of site use

When the research programme is completed at the site it is essential that the site is cleared of all markers and other artifacts. Unless required for future use any huts should also be removed. Remember that it is a requirement of the Antarctic Treaty that this activity will necessitate an Environmental Assessment. If it is decided that a hut should be left in situ, arrangements must be made for its maintenance. It is not acceptable to abandon a site without restoring it, as far as is possible, to its original state.

3.4 PREVENTING INVASIONS BY ALIEN SPECIES AND TRANSFER OF NATIVE SPECIES BETWEEN SITES.

(*Dana Bergstrom*)

3.4.1 Introduction of alien pecies

The risks of introduction of alien species to the Antarctic and to the Sub-Antarctic islands, while undoubtedly lower than in other ecosystems, are significant, as are the chances of previously naturalised species becoming major invasives (Frenot et al., in press). Although most Sub-Antarctic islands and some maritime and continental Antarctic ice-free now have alien taxa, the number of introduced species in these areas is much lower than in more temperate regions.

Antarctic scientists possibly have a higher chance of carrying alien propagules to Antarctica and Sub-Antarctic ecosystems than other Antarctic travellers because their work often takes them to alpine or northern polar habitats. Here they are likely to inadvertently pick up diaspores on clothing, equipment and equipment cases (Whinam, Chilcott and Bergstrom, in press). If these items are then taken to the Antarctic without being cleaned/-sterilised to remove or kill the diaspores, the opportunity to transfer such material to Antarctic ecosystems is created. The ecological potential for establishment of northern polar or alpine taxa is great, as such species have pre-adaptations to cold environments.

3.4.2 Between location transfer of species

While some form of quarantine measures are in place by most nations for travel to Antarctica and the sub-Antarctic, the application of quarantine procedures between major ice-free locations is not common. The implications of human transfer of taxa between locations can range from the modification of genetic structure of populations to changes in local biodiversity and subsequent flow-on effects on community dynamics. Such accidental movement of indigenous biota would also compromise scientific studies of molecular adaptation, regional evolution and biogeography, and reduce the inherent value that Antarctica offers as a model scientific system with limited anthropogenic influence.

3.4.3 Code of Conduct

RiSCC offers to it members the following code of conduct to minimise the chance of introduction of alien taxa to Antarctic and Sub-Antarctic environments and to reduce the risk of accidental transfer of taxa between major ice-free localities:

3.4.3.1 Risk assessment

As part of the fieldwork planning process the following risk assessment questions have to be answered:

- Has any equipment/ equipment cases/ field clothing/ boots, planned for use in the Sub-Antarctic /Antarctica been used in other natural environments, particularly alpine or polar environments?
- What are the means needed to clean this equipment/ equipment cases/ clothing/boots, and are these available?
- Will the field party be visiting more than one major locality?
- If yes, how will the field party ensure that equipment/ equipment cases/ clothing/boots do not carry diaspores between sites?

3.4.3.2 Field work

The following recommendations are made with regard to fieldwork:

Field planning

- If fieldwork requires moving between major ice-free localities, aim to conduct fieldwork in low diversity localities before high diversity localities.

Equipment

- When designing field equipment, reduce the capacity of the equipment to carry additional material and make the equipment easy to clean and sterilise.
- If equipment can not be cleaned effectively,

do not use this equipment between major localities but take multiple sets of equipment (eg planktonic nets).

- Be aware of where equipment cases are stored and that these cases do not accumulate dust or invertebrate infestations.
- When cleaning items, be particularly vigilant in removing soil, seeds and bryophyte propagules (including leaves).

Outdoor clothing and boots and packs

- If clothing can not be cleaned with bleach or similar compound, take new clothing/boots and packs. Be aware that items with Velcro can collect seeds (Whinam, Bergstrom and Chilcott, in press). Chose items with minimal or no velcro.
- Clean field items between sites. Be particularly vigilant in removing soil, seeds and bryophyte propagules (including leaves).
-

3.4.4 References

Frenot, Y., Chown S.L., Whinam, J., Selkirk P.M., Convey, P, Skotnicki, M., Bergstrom, D.M. (in press). Biological invasions in the Antarctic: extent, impacts and implications. *Biological Reviews*

Whinam J., Chillcot, N., & Bergstrom, D.M (in press). Subantarctic hitchhikers: Expeditioners as vectors for the introduction of alien organisms. *Biological Conservation*

4 Biodiversity studies

4.1 OBSERVATION AND COLLECTION LISTS

(Dana Bergstrom)

For many areas in the Antarctic basic information on the occurence of species is very incomplete. One of the aims of the RiSCC program is to develop a picture of biodiversity in the Antarctic biome. It is therefore suggested for all researchers involved in RiSCC to make a concerted effort to collect as much basic information as possible. This information will be collected into the RiSCC biodiversity database, which is developed and hosted by the Australian Antarctic Data Centre.

The following data should be recorded when making observations or collections for inclusion in the RiSCC Biodiversity database

Identifier (observer + observation or collection number)

- Date-Time

Site details

- Geographical position
- Lat/Long or UTM
- Locality determined by map or GPS
- SCAR Gazetteer place name
- General description
- Size of studied area
- Altitude
- Depth (in case of aquatic habitats)
- Distance from coast
- Slope / Aspect
- Substratum type (rock, soil, gravel, peat, etc.)
- Vegetation cover (%)

Species

- *Invertebrates*
  - Species names
  - Presence/absence or abundance per area
- *Vascular plants, bryophytes, and lichens*
  - Species names
  - Presence/absence or abundance (% cover or Tansley scale)
- *Microbial*
  - Presence/Absence or Abundance

Annex 1 provides a form for use in the field.

For details on how to enter data into the RiSCC Biodiversity Database see the AAD website at http://www-aadc.aad.gov.au/

Please, check the RiSCC website and/or the RiSCC listserver for the most recent requests for making collections, requested by RiSCC partners.

Observe always the rules of the station where you are working with respect to making collections. Make sure that you comply correctly with the legal regulations with respect to export of biological material from Antarctica and import into the country of the partner making the request.

4.2 TOWARDS A STANDARDIZED TAXONOMIC APPROACH FOR RISCC BIODIVERSITY STUDIES

(C. Ellis-Evans)

There is reasonable agreement on the taxonomic basis for higher plants and macro-invertebrates but there are still issues relating to the taxonomic criteria for certain groups of micro-invertebrates, certain cryptogams and, most particularly, micro-organisms. The concept of microbial species is a subject of continuing debate and the advent of molecular techniques has not proved a total answer and indeed has prompted even more questions and uncertainty in some cases. Given the importance of cryptogams, micro-invertebrates and microbial groups to RiSCC and our intention to put the biodiversity data in a database it is clearly appropriate that some guidance on taxonomic issues be provided to the RiSCC community to try to ensure consistency.

Acknowledged experts have been approached to suggest identification keys and/or species lists for specific groups of organisms and we would encourage everyone to use these suggestions which often relate to Antarctic specific guides. In some cases Antarctic-specific keys are not available and more general keys are suggested where appropriate.

This represents a first cut at this task and we will come back to the subject again early in 2004.

Groups covered below:

- Liverworts
- Lichens
- Mosses
- Protozoa
  - Ciliates
  - Rhizopods
- Gastrotrichs
- Tardigrades
- Crustacea
  - Copepods
  - Cladocerans
  - Ostracods
  - Rotifers
  - Nematodes

Liverworts

A definitive study of the liverworts present in the Antarctic has now been published and is backed up by an extensive herbarium collection.
Bednarek-Ochyra H, Vana J, Ochyra R and Lewis Smith RI. The liverwort flora of Antarctica. Cracow: Polish Academy of Sciences, 2000.

Lichens

A definitive account of Antarctic lichens has been recently published.
Øvstedal DO and Smith RIL. Lichens of Antarctica and South Georgia. A guide to their identification and ecology. Cambridge: Cambridge University Press, 2001.

Mosses

These will be comprehensively covered by the Illustrated Moss Flora of Antarctica currently being prepared by R. Ochyra and RI Lewis Smith. In the meantime, keys and description for many of the Peninsula mosses can be found in: **Ochyra R. The moss flora of King George Island, Antarctica. Cracow: W. Szafer Institute of Botany, Polish Academy of Sciences, 1998.**

Protists

(a) Ciliates
These are a particularly difficult group for non-specialists to identify. There are several systems in use and they change (sometimes substantially) within short periods of time. Most widespread has been the scheme of Corliss (1979), which has been regularly used in Antarctica in the past. However this is now somewhat out of date because a number of new taxa have been found in the meantime, others have been transferred to other taxa and of course many new facts are available regarding ciliate morphology/plasticity and biogeography. Some other more recent

taxonomic systems are not as detailed, e.g., go down to orders only, or are not well accepted by the community. It is therefore suggested to use the system of Lynn & Small (2002).
[**Lynn D.H., Small E.B. (2002) Phylum Ciliophora Doflein, 1901. In: Lee J.J., Leedale G.F., Bradbury P. (eds.),** ***The Illustrated Guide to the Protozoa*****. 2nd ed. Society of Protozoologists, Lawrence, pp. 371-656**]
This is modern and fairly detailed as it includes most (!) currently known genera. Thus, finding the taxonomic position of a given species should be relatively easy. In addition, this scheme is widely distributed and (hopefully) will be used for the next few years. Thus, it should be well suited also for the RiSCC database. Wolfgang Petz (petzwolf@i-one.at) has the scheme in digital form down to the families.

(b) Rhizopods
Another and more difficult problem is the system of the rhizopods. While the ciliates are a well-defined monophyletic group, the rhizopods are polyphyletic and their systematic affinities are not clearly resolved yet. Thus, their system is far less ' well-established' than in ciliates. There are several and often incomplete systems, i.e. classification schemes for one group only (e.g., naked or testate amoebae, or Filosea) and comprehensive systems are rare. In the above mentioned ' Illustrated Guide' there is also a system for the rhizopods. However, it is divided into groups — e.g. amoebae of uncertain affinities, order Arcellinida, ramicristate amoebae, testate amoebae with filopodia (group) etc. So there are no higher categories like ' class' and at least some of these groups are still not monophyletic. Their systematic position as well as their ' members' will possibly change considerably in the foreseeable future.

While this is OK for specialists and in taxonomic work, it is (probably) not so suitable for a database, which is also used by non-specialists. There should be a comprehensive and workable system with a clear hierarchical structure. It is suggested that RiSCC should use the higher-level system of Hausmann & Huelsmann (1996) for the classification of major groups like phylum (Amoebozoa), classes and subclasses. .
[**Hausmann K., Huelsmann N. (1996) Protozoology. Thieme, Stuttgart, 1-338]**
However note that a new edition is due out in 2003 which may change a few things!)

For the more detailed level of families and genera the schemes used in the corresponding chapters of the Illustrated Guide are suggested.
[Lee J.J., Leedale G.F., Bradbury P. (eds.) (2002) The illustrated guide to the protozoa. 2nd ed. Society of Protozoologists, Lawrence, 1-1432]
There is the possibility of providing a ' combined' system. Wolfgang Petz has the scheme of Hausmann & Huelsmann (1996) in digital form and could add the families from the Illustrated Guide where necessary.

The naked rhizopod section in the Illustrated Guide is rather heterogenous and instead the fine systematics of Page & Siemensma (1991) are recommended for the naked amoebae. The latter is based on pragmatic, easy to use keys and is well documented.
[Page F.C. & Siemensma F.J. (1991) Naked Rhizopods and Heliozoans. In: Mathes. D., Protozoan Fauna Bd. 2, Gustav Fischer Verlag, Stuttgart, New York, 297 pp.]

Gastrotricha

The taxonomy is currently unstable as there is considerable morphological variability in most freshwater chaetonotids but possibly the most up to date information on this group, including a key and morphotype diagrams can be found in:
[Maria Balsamo & M Antonio Todaro (2002). Gastrotrichia. In: S D Rundle, A L Robertson & J M Schmid-Araya (eds.) Freshwater Meiofauna — Biology and Ecology. Backhuys Publishers, Leiden. The Netherlands. p 45-61.]

Tardigrada

There is a very useful key to limno-terrestrial genera that includes all the current Antarctic forms.
[DR Nelson and S J McInnes (2002) Tardigrada. In: S D Rundle, A L Robertson & J M Schmid-Araya (eds.) Freshwater Meiofauna — Biology and Ecology). Backhuys Publishers, Leiden. The Netherlands. 177-216.]

Crustacea

There is a general listing of non-marine Crustacea for Antarctica and the Sub-Antarctic recently published by Pugh et al (2002) that is a very useful start point. Table 1 in this publication conveniently provides a higher classification of non-marine Crustacea showing the genera relevant to the Antarctic region whilst Tables 2 and 3 show their distribution.
[P J A Pugh, Dartnall, H.J.G and McInnes S.J. (2002) The non-marine Crustacea of Antarctica and the islands of the Southern Ocean: biodiversity and biogeography. Journal of Natural History, 36: 1047-1103.]

At a more specific level, we can consider copepods, cladocerans and ostracods.

(a) Copepods
Ian Bayly has revised *Boeckella* and *Pseudoboeckella* for South America and the Sub-Antarctic islands in **Chileno de Historia Natural 65. 17-63.** These genera are now fused into *Boeckella*.
B. Dussart & D. Defaye (1995) offer the most up-to-date keys for the fresh and brackish copepod genera.
[B. Dussart & D. Defaye (1995) Copepoda. Introduction to the Copepoda. In: H J Dumont (Coord. Ed.) Guides to the identification of the microinvertebrates of the continental waters of the world Vol 7. SPB Academic Publishing bv., Amsterdam]

(b) Cladocera
The Cladocera are a polyphyletic group with no taxonomic significance due to the lack of unifying characters. Within the Pugh et al. paper listed above, the Cladocera are attributed to the Order Anomopoda. A global list of identification sources is given in: **N.M Korovchinsky (1996) How many species of Cladocera are there? Hydrobiologia 321: 191-204.** The most up-to-date guide to the Cladocera is probably that by Smirnov (1996).
[Smirnov, N.N. (1996) Cladocera: the Chydorinae and Sayciinae (Chydoridae) of the World. In: H. J. Dumont (Coord. Ed.) Guides to the identification of the microinvertebrates of the continental waters of the world Vol, 11. SPB Academic Publishing bv., Amsterdam]

(c) Ostracoda
All the freshwater species fall within the order Podocopida. They can be difficult to identify but systematic information may be obtained from Bowman and Adele (1982).
[Bowman T E and Abele L G (1982). Classification of the Recent Crustacea. In: L G Adele (ed) The Biology of the Crustcea, Vol 1, Systematics, the Fossil Record and Biogeography. Academic Press, New York, pp 1-27.]
A good recent guide to the range of freshwater Ostracods is:
Meisch C (2000) Freshwater Ostracoda of Western and Central Europe. Susswasserfauna von Mitteleuropa 8/3. Spektrum Academic Verlag, Berlin, 522 pp.]
An illustrated key to South African ostracods is available in :
McKenzie KG (1977) Illustrated generic key to South African continental Ostracods. Annals of South African museum 74: 45-103.
and South American Ostracods are described in:
Moguilevsky A and Whatley R. (1995) Crustacea Ostracoda. In: E C Lepretto & G Tell (eds) Ecosystems de agues continentals. Metodologias para su studio. Ediciones Sur, Buenos Aires, p 973-999.

Rotifera

Although the systematics of this phylum are relatively stable there is an imbalance resulting from the emphasis on planktonic species and yet the benthic species are far more significant in terms of diversity in Antarctic and sub-Antarctic limnetic systems.
An extensive revision of rotifer taxonomy in the series "Guides to the Identification of the Micro-invertebrates of the Continental Waters of the World" is currently underway with four volumes published to date (since 1995) on the Lecanidae, the Notammatidae and Scariidae, the Proalidae and Dicranophoridae and the Ituridae. A simple key to family level is available in Volume 4 of these Guides, edited by Thomas Nogrady (1993). Specialized keys for the Bdelloidea (Bartos 1951, Donner 1965(in German)) and Monogononta (Ruttner-Kolisko 1974, Koste 1978 (in German)) complement the various general keys (e.g. Wallace and Snell 2001). The older references are given in the Guide.

[Wallace RL & Snell TW (2001) Rotifera. In J H Thorp & A P Covich (eds.) Ecology and Classification of North American Freshwater Invertebrates. Academic Press, New York.]

Herbert Dartnall has done some excellent work on Antarctic and Sub-Antarctic rotifers. He has published a detailed report on the rotifers of Signy Island and South Georgia

[Dartnall, H.J.G & Hollowday, E.D. (1985) Antarctic Rotifers. BAS Scientific Reports no. 100. 46 pp.]

Other references to more recent work on sub-Antarctic islands and continental sites are given in Pugh et al. (2002).

Nematodes

The systematics of the Nematoda are currently still in flux at lower taxonomic levels but most of the current literature is based around Lorenzen' s system (1981) - which is in German. Modern information suggests the case for dividing Nematoda into two classes, the predominantly freshwater Adenophorea and the predominantly terrestrial Secernentea. Considerable progress has been made with marine nematodes but it is still difficult to identify freshwater nematodes to species level.

[Lorenzen S. (1981) Entwurf eines phylogenetisches Systems der freilebenden Nematoden. Veroffentlichungen des Instituts fur Meeresforschung in Bremerhaven. Suppl. 7, 472 pp.]

There is an excellent chapter by Walter Traunspurger in Freshwater Meiofauna: Biology and Ecology. In Table 2 of this chapter he provides a relatively traditional classification of nematodes with references to appropriate keys whilst in Table 3 he outlines the characteristic features of the nematode orders which are also illustrated in Fig 2.

[W. Traunspurger (1981) Nematoda. In: S D Rundle, A L Robertson & J M Schmid-Araya (eds.) Freshwater Meiofauna - Biology and Ecology. Backhuys Publishers, Leiden, The Netherlands, p. 63-104.]

TERRESTRIAL SYSTEMS

5 Abiotic parameters

5.1 THE COLLECTION OF CLIMATE DATA

(Roger Worland, Manuel Bañón and Antonio Quesada)

Environmental data, in particular climate data, are collected on three scales:
Regional scale: 50 x 50 km
Meso / micro scale: 10 x 10 m
Nanoscale: 1 x 1cm

5.1.1 Regional Scale

Meteorological stations are a primary source of regional climatic information. If there is a meteorological station close to the RiSCC field site, this will provide basic meteorological data.
Otherwise it may be necessary to install an Automatic Weather Station (AWS) at the field site for general meteorological data collection.

5.1.2 Meso- / microscale

Meteorological data are of primary importance for the investigation of the terrestrial and limnetic ecosystems within the context of the RiSCC programme. Where (micro) meteorological data is required to characterise a particular research site it is recommendable that a dedicated automatic whether station (AWS) should be installed which can be programmed to acquire data at the appropriate frequency.

If the sampling area is to be used for several seasons it is recommended that the station should be designed to work continuously throughout the year, including the winter months. The meteorological variables that should always be recorded for comparison with other sites: air temperature, air humidity, wind speed and direction, radiation (global and PAR) and temperature at the soil surface. Unused channels can be utilised to record additional parameters specific to the particular study at the site e.g. conductivity, water depth and temperature at different depths.

In most cases the meteorological station will be required to operate under extreme environmental conditions i.e. low temperatures (-30ºC or lower) and very high wind speeds (150 km/h or more). It is also possible that the station will only be visited once per year in which case it should have a suitable power source utilising solar or wind power and have sufficient memory to store the data for at least one year. Where the station is installed at a remote site, weight may be an important consideration, particularly if it is to be manhandled over long distances. All these factors should be considered when the model of the station is chosen.

The methodology, makes and models described in this section are only those that have proved to be reliable in Antarctica. Many other manufacturers supply equipment, which may be equally suitable, and function with the same accuracy as the ones specified here.

Setting up a new station

The actual position where the AWS is to be located should be given careful consideration. It is very important that the chosen site is representative of the area to be investigated. It should not be positioned in an area which is more protected than the sampling site or where it will have an effect on the study e.g by causing a snow drift.

The site description should include a diagram, of the area and the following information:
- Location (GPS if possible)
- habitat

- logger type and serial number
- name and version of logger program
- date and time the logger was started, including whether logger records using local time or GMT (and if local time, give the difference between this and GMT)
- A list of the sensors including serial numbers and locations e.g. the height of the sensors above the soil surface - this needs to be a comprehensive description which does not rely on any background knowledge (explain any abbreviations used and do not just give details such as a cloche number but include a description of the cloche type and location)
- brief description of why the logger is being deployed.
- Names of the people involved (i.e. who programmed the logger, who set it up, who is responsible for down-loading the data, etc.)

Downloading data

Each time data are downloaded from a logger the resulting data-file(s) must be self-explanatory, allowing the data to be interpreted without relying on any additional documentation. The data-file should be in comma delimited ASCII format and consist of the following elements:

- logger type
- logger location
- name of logger program
- date the data were downloaded (must include the year)
- column descriptions for the data-file (with enough detail so that it is clear to what each column in the data file refers)
- any notes pertinent to the data contained in the data-file. If there are extensive notes these could be included in a separate file, preferably in ASCI text format, which is given the same file name but a different extension (e.g. data1.dat and data1.doc). This should include any problems with the logger that have been observed and what, if anything, has been done to rectify them.
- the data, in comma delimited ASCII format

Modifications

Any modifications which are made to the AWS, sensor locations or logger program must be fully documented. This documentation should be in ASCII text format and include:

- date and time the modification was made
- description of the modification stating clearly what was done, why it was done and what effect (if any) it has on the data.
- the name of the first data-file following the modification and, if the modification was made midway through a data-file, the day and time of the first logger recording after the change.

A basic automatic weather station

A basic station should include:

- Energy supply
- Logging unit
- air humidity and temperature sensors
- wind speed and direction sensors
- total radiation sensor
- soil temperature sensors in the active zone

Data should be measured at 10-minute intervals and averaged over each hour. However, the recording interval may depend on the specific application. Too much data rapidly becomes unmanageable.

Energy supply

The logging unit requires energy to work. In year-round stations the energy supply has to be continuous, although most units have non-volatile memory that maintains stored data even in absence of energy. Solar panels and/or wind generators can be used to maintain the charge in the batteries to allow continuous operation for at least one year. Specialised gel batteries are available which are designed to operate with solar panels and to operate at low temperatures. Most AWS are likely to require a 100 Amp hour battery but these are expensive and very heavy. In some cases the AWS may be able to operate directly

from a solar panel with excess power being stored in a smaller battery to maintain operation during hours of darkness. The exact design of the system will depend on the power requirement of the logger and the local environmental conditions including the severity and duration of the winter and the number of daylight hours available for charging the battery.

Logging unit

Since one of the RiSCC aims is to build a latitudinal gradient in the Polar Regions, it is important that the meteorological data is comparable between sites and along the gradient. It is therefore important that the loggers and sensors are reliable and of a high quality. AWS manufactured by Grant Instruments, Delta T and Campbell Scientific Instruments have been used by RiSSC participants and proved to be reliable but Campbell are probably the most robust and the most suitable for Antarctic conditions. The Campbell CR10X logging unit has been installed at several polar sites and has given excellent results, and the ratio quality/price is quite reasonable. It is important to ask for the enhanced electronics for extremely cold conditions (-55ºC).

This model contains 12 single channels or 6 differential channels, three excitation channels, 2 pulse channels and 8 digital I/O ports. The number of input channels can be increased with the addition of a multiplexer unit. The RAM capacity is important and should be calculated using the number of channels, the logging frequency and the time interval between downloading the data. 128 Kb is enough for running the programme and storing data, and at least 2 Mb is recommendable for storing the data (non-volatile). Some loggers, such as those supplied by Campbell Scientific, can utilise external memory, which simplifies downloading the logger and greatly extends the amount of data that can be stored. 2 Mb PCMCIA cards are normally adequate.

The data logger and the external memory unit should be installed in a weatherproof box with desiccant to absorb any moisture present in the air when the box is closed. Loggers are normally mounted above snow level on strong, metal tripods, which also provides support for some of the sensors and the solar panels. This structure must be very robust and held in place with guy ropes especially in polar regions experiencing high wind speeds. Large batteries are more appropriately housed in a separate enclosure at ground level where they may be insulated from low air temperature by snow cover.

Measuring protocol

When designing the measuring protocol it is important to consider the energy available and the total amount of memory built into the system Normally, readings should be taken from each sensor every 10 minutes and stored with the time (year, day, hour and minute). Records should include:

- air temperature and humidity,
- air temperature at the soil surface,
- integrated radiation (PAR and global) in the previous 10 min,
- wind speed and direction at the measuring time and the maximum speed and direction at that moment during the previous 10 min.

At the end of the day we can obtain the mean temperatures and humidity during the day, the maximum and minimum values and times when this was measured, global and PAR radiation integrated over the day, mean wind speed and direction and maximum value for wind speed and its direction and time. Other parameters as voltage or internal temperature of the logging unit might be interesting, if memory is available. All these parameters can be calculated within the software running a Campbell Scientific data logger.

Air temperature/Relative Humidity

Temperature and humidity are often measured with a combined sensor mounted in a ventilated radiation shield. For "standard" measurements the sensor should be mounted between 1.2 and 2m above the ground.

The humidity element should have a linear output and should be capable of operating at sub-zero temperatures (e.g. Rotronic hygrometer C-19). Data is normally recorded as %RH with a range from 0 to 100%. This sensor should have a reproducibility of <1% RH and a typical long term

stability of 1% over 12 months. The calibration should ideally be checked at least annually, using saturated salt solutions.
Air temperature should be measured using a platinum resistance sensor (e.g. PRT 100) with a range of at least -40 to +30°C. Reproducibility of such sensors should be better than 0.1°C with a time constant of < 15 sec. Temperature calibration should be stable for several years.

Air temperature at the soil level

This is a particularly interesting parameter to be measured in the RiSCC context, since this temperature is more similar to that experienced by our terrestrial organisms than the standard meteorological air temperature. It also needs a meteorological shelter and typically is deployed 10 cm above the ground level. As this sensor is presumably going to be covered by snow, and may be very close to the water level, it is important to purchase a waterproof sensor.

Wind speed/Wind direction

The anemometer should be installed 3 m above ground level, but if this is impractical, try to obtain the highest position (at least 2.5 m). This instrument has to be robust enough to withstand wind speeds of over 150 km/h and have a resolution of 0.3 m/s at low speed or 1 m/s at high speed. The wind direction should have a resolution of 5 to 10º. Anemometers tend to be more problematic than other field sensors as snow and ice can block the propeller or other moving parts causing loss of data. Anemometers can be purchased with heating systems. Solar powered stations, however, are unlikely to be able to provide enough energy to run them, especially in winter, when the problem is most likely to occur. If this option is to be considered, the energy requirements must be carefully calculated to avoid malfunctioning of the AWS. For non-heated instruments, the 05103 sensor (R.M Young) is a good choice.

Radiation

For energy budget calculations a global radiation sensor is required to enable heat exchange calculations. However, from the biological point of view, PAR (photosynthetic active radiation) sensors are very useful as well, since they provide information on the radiation available for driving photosynthesis both in terrestrial and aquatic organisms. For the global radiation we can chose a pyranometer (305-2800 nm), with a resolution close to 5 W/m^2. The Kipp-Zonen CM5 is a good example. For the PAR sensor we need to purchase a flat sensor 2□ corrected. Both sensors have to be waterproof and should operate in the range of temperatures we are going to find.

Precipitation

Possibilities are:

- tipping bucket rain gauge with an antifreeze reservoir
- Pluvio system by Ott (however, this system needs 12 V power).

Various units are available but those with heaters can be expected to use large amounts of power, which are not suitable for most remote stations. The Antifreeze-based design (Mc Caughty and Farnes, 1996) is cumbersome and prone to time delays as snow is captured in a reservoir of antifreeze liquid and may take up to an hour to melt and overflow into the tipping bucket mechanism below. However, it is cheap and uses no extra power. Data should be given in mm/hour.

Water availability

Several sensors based on time domain methodology are available to derive water content information. Examples area the CS615 water content reflectometer supplied by Campbell Scientific with 2 x 300 mm long probes, the TRIME-ES supplied by IMKO with 2 probes as short as 50 mm, and the Delta-T Devices *ThetaProbe* with 3 probes of 60 mm. All provide information on % water content.

Snow cover thickness and duration

Options include:

- Sonic ranging sensors (e.g SR50 marketed by Campbell Scientific) are reliable with an accuracy of 0.1mm although 1 cm resolution may be enough, for automatic recordings.
- Temperature sensors mounted at various heights on a mast. Temperature variation of each sensor is logged, the theory being that once buried in snow the variation is less than when exposed to air. The British Antarctic Survey (BAS) has a design for modular sensors. Each module houses 4 sensors mounted 2.5 cm apart. These can be stacked on a mast to give the required depth of coverage. By design, the best resolution is 2.5 cm. This type of system seems prone to ablating-out giving false readings.
- BAS has also developed a digital camera system in an environmental housing which monitors a matrix of snow stakes to allow depth and percentage area covered to be derived from photographs using image analysis. The system will record an image each day for over a year and has proved reliable at temperatures down to -35 °C.

5.1.3 Nanoscale

(Leopoldo García-Sancho)

Climate is measured at nano-scale to provide information on actual conditions experienced by organisms in the field.

Equipment

Portable, battery operated data loggers, for example:

- Squirrel 1200 Series (Grant Instruments, UK) measuring 12 input from different sensors (4 temperature, 4 PAR, 2 humidity).
- Squirrel 1000 Series (Grant Instruments, UK) measuring 16 input from different sensors. General features:
  - Easy to set up using buttons or display on logger or by PC
  - Very low power consumption enables record measurements for long periods in the field without changing batteries
  - Alternatively they can be connected to external power supply
  - Memories to store up to 64,000 or 260,000 measurements which can be expanded to 500,000.
  - Highly accurate $\pm$ (0.1% of readings, +0.2% of range span)
  - Environmental operation: -30 to +65ºC, 0 to 95%RH
  - Communications via RS232C at different baud rates

Temperature sensors:

- Thermistor type FMSU (Grant, UK). Range —50 to 125ºC, Resolution 0.2ºC. diameter 0.6 mm.

Humidity sensors:

- Type HMP35 (Vaisala, Finland). Range 0 to 100%, Resolution 2% (0-90%), 3% (90-100%).

PAR sensors:

- SKP type (Skye, UK) or Licor (Licor, USA). Range 0 to 50 mV (1mV/100 $\mu mol.m^{-2}.s^{-1}$), resolution 50 µV.

Procedure

Micro- / nano-climatic data of the selected sites can be recorded at different programmed time intervals depending on the purpose of the investigation. Event inputs at very short intervals (as small as 2 sec) are recommended to accurately track plant nanoclimate under varying weather conditions. When comparing different exposures/altitudes/latitudes a larger event input interval or an average input interval can be selected. Temperature sensors can be inserted into the lichen laciniae (fruticose thalli) or vascular plant and bryophyte leaves or beneath the lower surface of foliose thalli. Humidity probes and PAR sensors should be placed in the vicinity of the plants, the latter always matching the plant orientation to light interception. Air temperature must also be recorded at ca. 1 m above ground level. The use of terostat (Terostat IX, Henkel Teroson, Germany) is highly recommended for fixing sensors and cables as it has high resistance to extreme weather conditions.

Different microclimatic investigations can be carried out: altitudinal gradients or different slope

exposures for a selected species, microclimate of different species within a defined plant community, microclimatic study coupled with ecophysiological investigations, e.g. photosynthetic activity through chlorophyll fluorescence measurements.

5.2 SOIL DESCRIPTION

(Yves Frenot)

5.2.1 Introduction

The soil characteristics, with climatic conditions, are some of the most important factors influencing plant and soil fauna communities. For this reason these characteristics should be considered in most terrestrial studies along the AEG. The detail of the soil description differs according to the level of approach. At the basic level information on the nature of the soil (organic, mineral), its depth and the overall soil moisture may be quite sufficient (no special form is needed; this information is included in plant community form). At the advanced level, or when permanent plots are set up, a more precise description of the soil environment is recommended.

In order to help people who are not familiar with soil science, we provide a standardized form (see Annex 4) to describe soil profiles. This form is not made for exhaustive pedological studies, but presents a restricted set of parameters that will help to answer many of the questions posed in the RiSCC programme and which may be easily determined in the field.

5.2.2 Minimum equipment

- Shovel
- Knife
- Tape measure
- Munsell soil colour chart
- Plastic bags for samples

Optional: metallic cylinder to collect known volume of undisturbed soil samples

5.2.3 First stage : digging a pit

- *How to choose the site?* very close to the permanent plots (few metres) or the core sites, in a similar topographical and physionomic situation
- *How deep has the pit to be?* depends on the objectives. If the study is to assess the soil processes, the best depth is when the rock (or permafrost layer) is reached; if the study is only related to a plant study, digging may stop where the roots disappear.
- *Should we use an auger?* not suitable for soil description : use an auger only to verify some characteristics that have emerged from the soil profile description, or to collect some small samples of recognizable horizons, or to estimate the total depth of the soil.
- *Can we use natural profiles (e.g. a river bank)?* Yes, if close to the permanents plots. In that case, just refresh the soil profile. No, if not representative of the study site.
- *How to clean the profile?* use a knife to refresh the profile, starting from the top to the bottom.

5.2.4 Soil characteristics to describe : 10 points (intermediate level, Annex 4)

The unit of description is the horizon. When the soil profile is clean, the first stage is to identify the different horizons on the basis of changes in colour, texture, structure or pedological traits.

Diagram

The space on the left part of the form is to draw a diagram of the profile. It supplies information about depth, number of horizons, and possibly on the shape of the lower limits (straight, undulated, interrupted, etc.)

Depth (cm)

Two values characterize the depth of a horizon: the top and the bottom, with the surface of the soil as reference. If the lower limit of the horizon is undulated, min and max depth may be provided. The zero was formerly defined by the limit between holorganic and organo-mineral layers but it is doubtful for peat. So, the zero level is now usually taken just below the litter layer (Oi).

No of horizons

Horizons are numbered from the top to the bottom. At the end of the description, pedogenic processes may be inferred from the macromorphological traits and it is possible to refer to each horizon as recognized types (A0, B, Bt, C, etc.). However, this stage is not compulsory.

Colour

This is one of the most important traits of a soil and numerous elements may be inferred from this parameter, for example:

- presence, relative abundance, leaching or accumulation of organic matter, iron, manganese, clay
- reduced vs oxidized iron due to anaerobic vs aerobic conditions
- water dynamics (in relation to iron and manganese dynamics)

In the field, the observation usually describes moist horizons; if possible, colour of dry samples can be taken in the lab after drying. The colour of a horizon is usually the colour of the matrix, *i.e.* that material which has the highest cover on the face of the profile. The colour of other material, referred to as pedological traits, is listed separately. The use of the Munsell soil colour chart is essential to make the information understandable for everybody.

Soil moisture

For this parameter, the most important is to provide comparisons between horizons.
5 classes are commonly used:

1. dry: no detectable moisture
2. slightly moist
3. moist : soil sample is malleable, water content is close to field capacity, no free water
4. very moist
5. saturated: the whole porosity is water saturated or the layer is under the water table

Organic matter content (OM)

Description of the organic material is rather difficult for a non-specialist. It refers to the nature of organic material, its structure, colour, consistency, degree of link with the mineral material, distribution inside and between aggregates, etc. In the basic soil description we have only included information related to the relative abundance of the organic matter in the different horizons, using the following classes:

- no organic matter (0 %)
- little organic matter (< 2%)
- medium organic matter content (2-10 %)
- high organic matter content (10-15 %)
- very high organic matter content (15-25%
- peaty soil (> 25%)

The organic matter content may be determined quantitatively by soil analysis.

Coarse elements

Observations are made on the quantity of each size class in the soil in each horizon:

- Block (>20cm)
- Stone (5 - 20 cm)
- Pebble (2 - 5 cm)
- Gravel (2 cm - 2 mm)
- Fine soil (< 2 mm)

Content is expressed in % (see figure 5.1). The sum should be 100.

Texture

Texture concerns only the fine soil (< 2mm) and refers to the grain-size distribution in this fraction. For a non-specialist this is difficult to evaluate in the field. The most important is to note the dominant fraction(s) in each horizon.

- Sand 0.2 - 2 mm
- Silt 0.2 - 0.002 mm
- Clay < 0.002 mm

This can also be quantified with soil analysis.

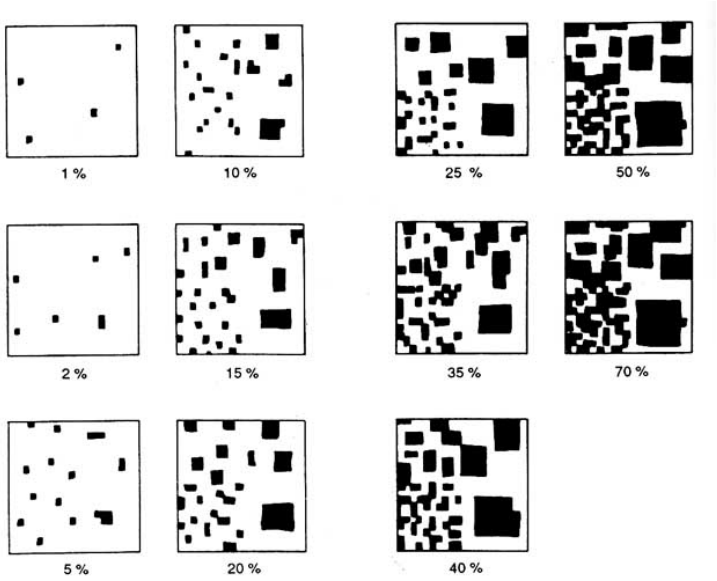


FIGURE 5.1 *Visual estimation of percentage coverage according to the size of elements (Folk, 1951).*

Structure

The role of soil structure in water retention and in capacity of rootlets to exploit the soil resources is extremely important. The presence of aggregates and their size must be noted, using to the following classification (see figure 5.2).

Note: this list is for mineral and organo-mineral horizons, not for holorganic horizons.

- no aggregates present
  - horizon not coherent = particular (sand for example)
  - horizon coherent = massive

- aggregates present
  - rounded
    - ± spherical, low porosity, curved surfaces = **granular** (1)
    - high porosity, curved and flat surfaces; irregular, ± grouped together = **grumous** (2)
    - grumous structure with aggregates < 1 mm = **fluffy**
  - angular edges and/or flat surfaces
    - predominantly horizontal, large width and small height, angular edges = **lamellar** (8)
    - predominantly vertical, flat surfaces and angular edges = **prismatic** (5)
    - prismatic but with a rounded summit = **columnar** (6)
    - flat surfaces, few in number, similar in area = **cubic**
    - predominantly oblique, flat surfaces, sometimes curved, smooth or striated = **platy** (7)
    - (structure observed only in Vertisol ; probably not encountered along the AEG)
    - no dominant direction, flat surfaces, angular edges = **angular polyhedral** (3)
    - different types of surfaces and edges, edges often blunt = **subangular polyhedral** (4)

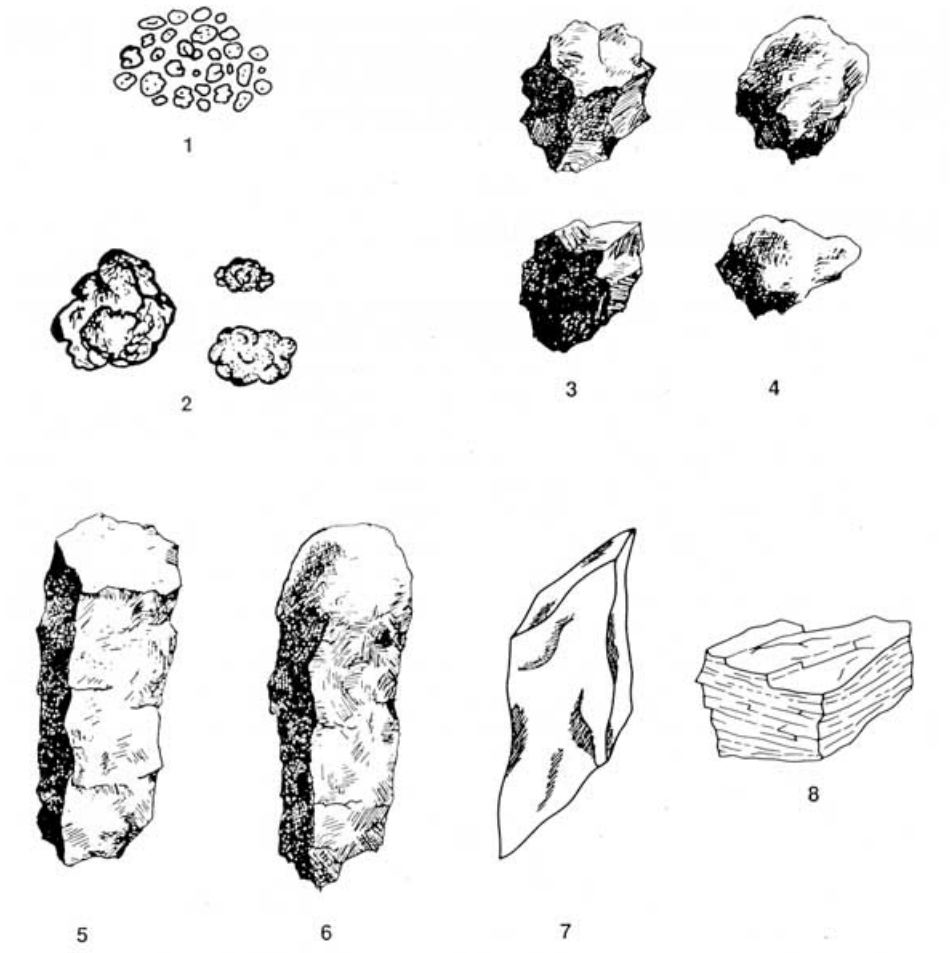


FIGURE 5.2. *Shape of aggregates in the main soil structures (from* Soil Survey Manual*, 1951, U.S. Department of Agriculture).*

Roots

As for soil moisture or organic matter content, the aim of this field is to provide comparisons between horizons and to give information on the location and size of roots.

size classes (alternatively terms such as very thin, small, large, etc. may be used) :

- <1 mm
- 1-2 mm
- 2-5 mm
- 5-10 mm
- 10-20mm
- > 20 mm

abundance classes (estimation by counting the number of roots in 10x10 cm areas; alternatively symbols such as +++, ++, +, 0 may be used to give relative abundance between horizons only)

- no roots (0)
- very few (1 - 15 / dm²)
- few (15 - 75 / dm²)
- numerous (75 - 200 / dm²)
- very numerous (> 200 / dm²)

health :

- healthy
- with necrosis
- decayed
- with rusty crust

Particular features / pedological traits

The most common pedological traits contributing to the heterogeneity of the horizon material are:

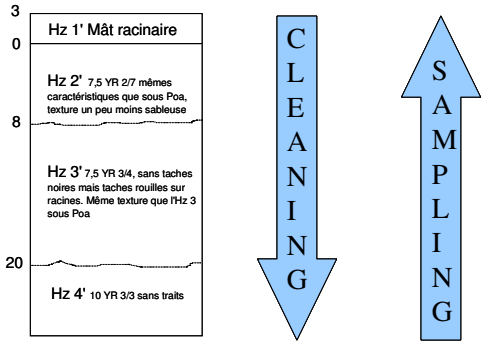
- *oxidation*: oxidation of iron and/or manganese; may be associated with chemical weathering, not necessarily with water-logging
- *reduction*: they are visible only when the profile is fresh and they disappear in a few hours
- *degradation*: related to clay dynamics
- *decoloration*: usually light spots related to iron eluviation
- organic matter
- other horizon

When a trait is described, it is useful to note its colour, its cover (%) and, if possible, its shape.

5.2.5 Sampling

After describing the soil, the profile must be refreshed with a knife from the top to the bottom. Then, samples of each horizon are collected from the bottom to the top (in order to avoid pollution of samples by the upper horizons).

N.B.: a standardized sampling on the basis of depth for different soils is nonsense! Every soil sample should be related to a specific horizon.



Samples for analyses

For soil analysis the fine particle fraction (< 2 mm) is used, after drying. Make sure that you have enough material for these analyses: a minimum 500 g of fresh soil without pebbles and stones is recommended. but if the coarse elements are abundant or if the soil is very moist, the weight of the sample may be higher (1 or 2 kg). Transport the samples in polyethylene bags or cardboard boxes, double labelled.
Samples are dried as soon as possible. Crumble the samples with your hand, spread them on a sheet of paper, and air dry them, or dry them in an oven at a temperature < 50°C.

Samples for water content, bulk density and porosity measurement

Use a metallic cylinder, 5 cm diameter, 3 to 5 cm height. The objective is to collect an exact volume of undisturbed soil sample.
To measure porosity the soil must be kept in the cylinder during transport. If only water content and/or bulk density are to be measured, the sample may be transferred from the cylinder to a plastic bag immediately after its collection.

5.2.6 Some remarks on soil moisture evaluation in ecological studies

When studying the climate change impact on plant or soil fauna communities, attention must be paid to two particular features :

- *soil temperature*, studied using probes connected to an automatic weather station (see the section on climate measurement)
- *soil moisture*, and more precisely, water availability for plants.

This second point is very important, at least in the subantarctic islands.

The easiest method to assess the water content of a soil (or a horizon) is to weigh fresh and dry samples. This measurement may be repeated over time and provides a good idea of the moisture regime of a soil. However, this parameter is not suitable to evaluate the water availability for the plants, which is the most interesting point to assess in such studies. The relationship between water content and water availability depends mainly on structure and texture of the soil. It is possible to model this relationship for each soil type (or soil horizon) :

Pressure plate extractors are used in such an approach. . This requires expensive equipment, and is time consuming. Therefore it is not realistic to systematically recommend its use in

the RiSCC programme. However, for permanent plots such calibration could be done with the help of soil analysis laboratory. Subsequently only knowledge of the water content (and the way it changes over time), is necessary to determine the water status in a soil at a given time, and the water availability for plants

Assessment of soil water content may be obtained either by weighing, following the following equation (expressed in %):

$$((Wf - Wd) / Wd) \times 100$$

where *Wf* is the fresh weight and *Wd* the dry weight (oven-dried at 105°C up to stable weight). It is important to express this water content in relation *Wd* (and not *Wf*) for comparisons between soil types. Sometimes this ration may be >100 %, especially in moist organic soils or peats.

Another method to measure soil water content is by the use of special probes, connected to an Automatic Weather Station (AWS), when one is available close to the study site. Numerous probe types exist. They generally need calibration before use. They are usually sufficiently accurate for the monitoring of the soil water content in ecological studies. If pF / water content curves are available for each permanent site, this is the recommended method.

One type of probe, called a tensiometer, using a porous ceramic cup, provides information on the soil water status directly in terms of water potential. However, the use of these probes is difficult in the Antarctic or Sub-Antarctic, due to their fragility (a problem in mineral soil with many coarse elements) and the necessity to fill them with antifreeze solutions, which may alter the results.

Addresses of suppliers and more technical information on soil water assessment and description of soil profiles may be obtained from Yves Frenot (yves.frenot@univ-rennes1.fr) on request.

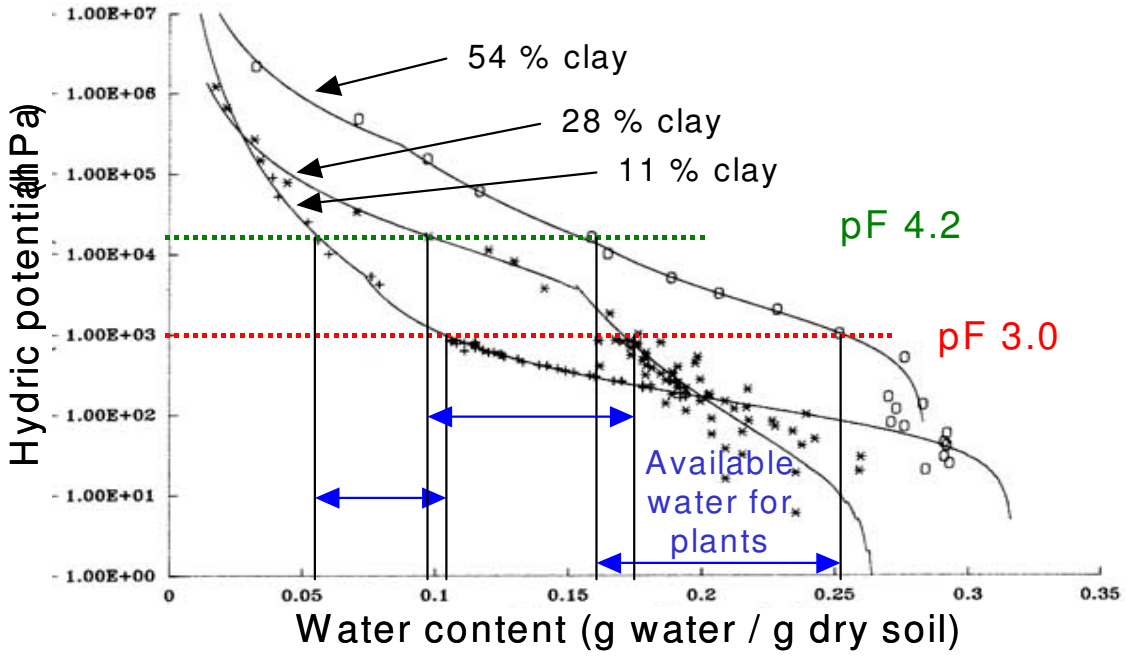


FIGURE 5.3. *This figure illustrates, for 3 different soil types, the relationship between the water content (X-axis) and the hydric potential (Y-axis), i.e. the pressure needed to extract the water from the soil porosity (expressed in hPa or in its logarithmic form, named pF). The water available for plants is usually defined by two limits: the field capacity (pF = 3.0) : all the macroporosity is full; the permanent wilting point (pF = 4.2): above this point, no water is available for plants.*

5.3 THE ASSESSMENT OF SOIL DISTURBANCE BY FROST ACTION

(Jan Boelhouwers)

5.3.1 Objectives

Freeze-thaw cycles in sediments with adequate moisture can result in cryoturbations that negatively affect vegetation colonization and survival, influence soil development through mixing and nutrient redistribution and enhance soil loss. As soil disturbance is highly temperature and moisture dependent the process is highly responsive to climate change. The objective of this protocol is to provide non-technical guidelines for the easy assessment of the extent of soil disturbance and the rates at which they operate.
The measurements are relevant to environments dominated by daily and seasonal frost cycles and are most applicable for the Sub-Antarctic islands. It is important that observation sites should be located as close as possible to an AWS or data logger recording soil temperature profiles.

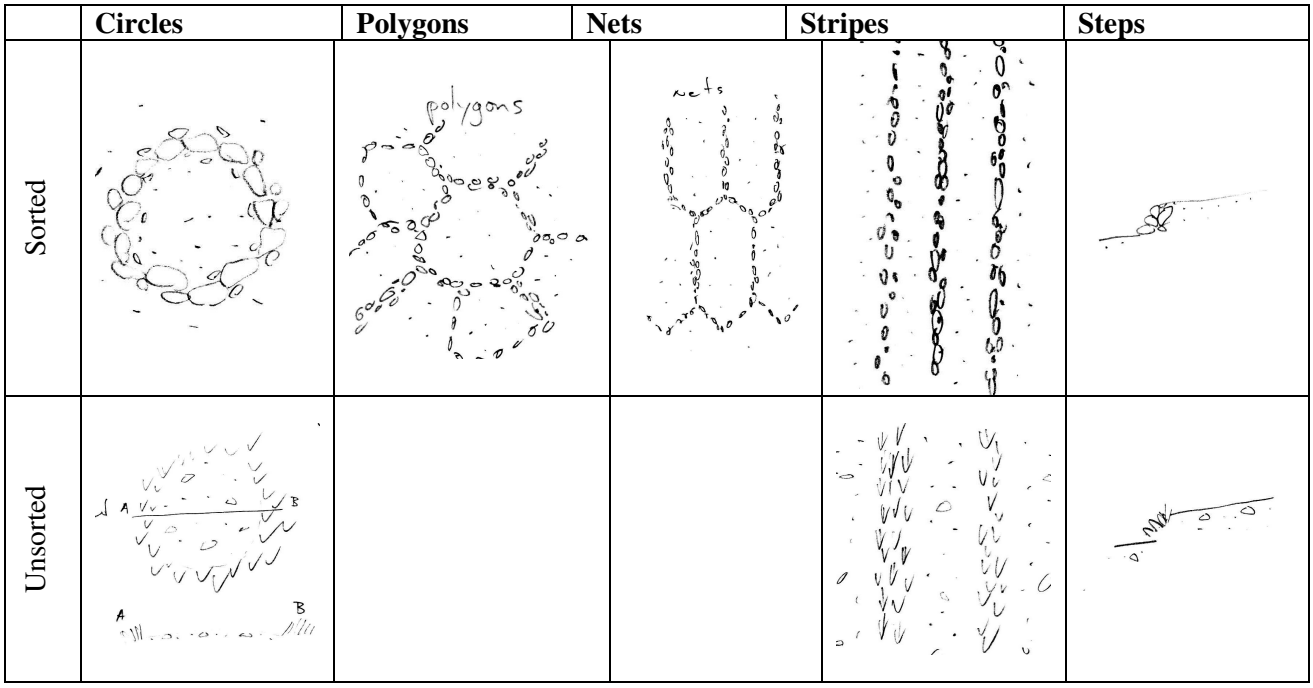
5.3.2 Morphological description of soil disturbance features

Sedimentary structures by soil frost disturbance are collectively known as patterned ground. The presence or absence of these forms and their size provides in an indication of the extent of soil frost disturbance at a site.

Classification of patterned ground
Patterned ground may be sorted or unsorted. Sorted forms have a coarse stone border with a centre of finer sediment, unsorted forms lack this distinction. Figure 5.4 pictures the various types of patterned ground that may be found. Classification of patterned ground is well described by Washburn (1956). The Southern Hemisphere Working Group of IPA is presently developing a website with an image library, to illustrate examples of these and other permafrost landforms.

Size of patterned ground
Size of patterned ground increases with severity of frost and can provide a good proxy for frost intensity. Parameters that define the morphometry of the patterned ground are provided in Figure 8.

FIGURE 5.4. *Morphological classification of patterned ground (terminology based on based on Washburn, 1956).*



5.3.3 Assessment of depth of soil disturbance

Depth of active soil frost disturbance can be assessed by describing the depth to which larger stone fragments are heaved out of the soil in patterned ground (depth of vertical sorting). In addition, sorted patterned ground will display a lateral sorting of sediment by particle size, indicated by a depth of lateral sorting. The depth of lateral and vertical sorting should be described as defined and illustrated in Figure 5.5.

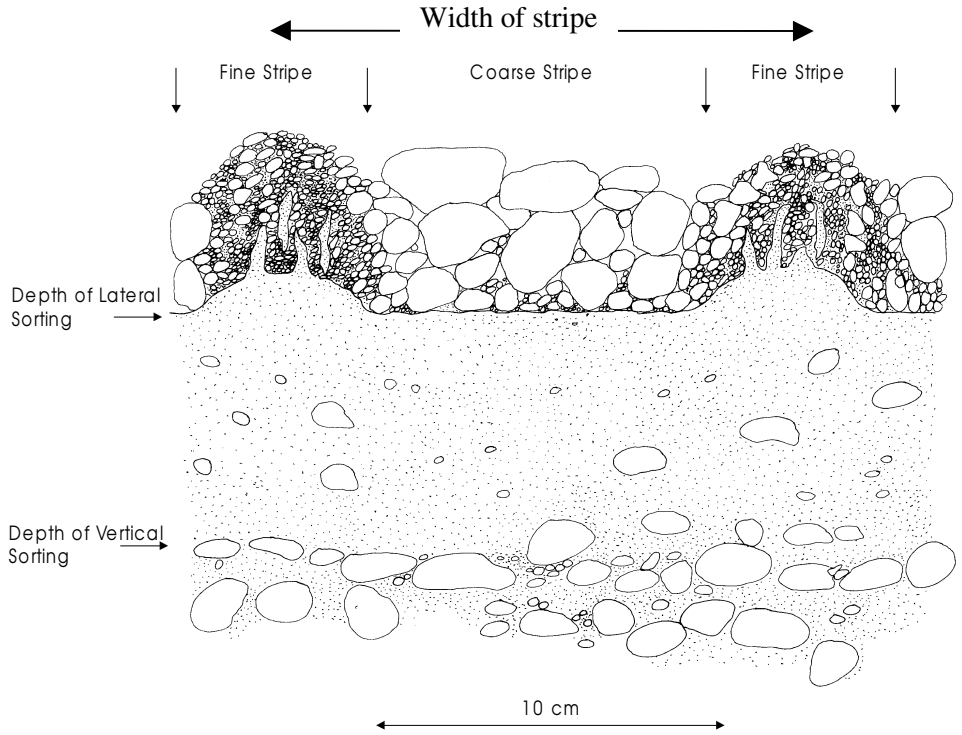


FIGURE 5.5. *Definitions for describing the surface morphometry of patterned ground, exemplified for a sorted stripe from Marion Island. Depth of effective frost penetration in the soil is indicated by the depth of vertical sorting (from Holness, 2001).*

5.3.4 Assessment of rates of soil disturbance

The morphological features described may be relict (result of past soil frost activity) or active (currently forming under present-day climate). The extent of current activity can be assessed by a simple and inexpensive method using wooden dowels.

Wooden dowels inserted at right angles to the ground surface will record the vertical heaving activity by soil frost processes down to the depth of insertion. Periodic checking of the extent of elevation of the wooden dowels above the surface provides an indication of the rate and extent of frost heave activity. Complete heave of the dowels out of the soil may be achieved within 10 daily freeze-thaw cycles in the Subantarctic or take over a year in seasonally freezing and thawing sediments.

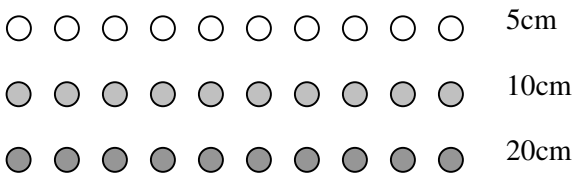
Site selection
The site should be located where patterned ground is present, preferably on a near-horizontal surface. The site should be free of vegetation. Sites should be located as close as possible to an AWS or data logger recording soil temperature profiles.

Materials
Round wooden dowels with a diameter of ca. 5mm, should be cut to lengths of 5cm, 10cm, 20cm, 30cm, etc. The longest dowels should at least reach the depth of vertical sorting described above. Per monitoring site a minimum of 10 dowels of each length should

be installed.

Installation of dowels
Dowels of equal length should be inserted in a row with 10cm spacing between each dowel. The pegs should be carefully pushed into the ground at right angles to the soil surface. Avoid creating gaps at the sides. The pegs are inserted until level with the ground surface. The result will be an array as shown in Figure 5.6. Avoid trampling the site as compaction of the soil affects heave rates!

Measurement
Periodic measurement of the length of the dowels above the soil surface is required. Complete heave of the entire length of the dowel will result in the dowel resting flat on the surface (Figure 4). Dowels inserted beyond the maximum depth of soil frost penetration will not heave.



Etc. Rows continued at 5 or 10cm intervals to beyond depth of vertical sorting at the site.

FIGURE 5.6. *Dowel array for the monitoring of rates of frost heave and maximum depth of frost penetration. Distance between dowels is 10cm. Minimal distance between rows is 10cm.*

5.3.5 References

Holness SD (2001). The orientation of sorted stripes in the maritime subantarctic, Marion Island. *Earth Surface Processes and Landforms* 26 (1): 77-89

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5.4 ACTIVE LAYER MONITORING PROTOCOL FOR ANTARCTICA

(Mauro Guglielmin)

5.4.1 Introduction

The active layer is the zone above permafrost that is subject to seasonal freezing and thawing. The same term is sometimes used to define the layer of ground above the maximum depth reached by 0°C isotherm (French, 1996). For the ecologist the first concept is more appropriate, because the crucial point is the presence or absence of liquid water, which conditions biologic, pedologic and biogeochemical processes.

The monitoring of the active layer thickness records the seasonal and annual variations of ground surface temperature and can be undertaken with a relative simple technology and good accuracy (Guglielmin and Dramis, 1999; Nelson et al., 1998).

The ground surface temperature (GST) reflects the net energy balance of the surface, which depends on several climatic factors such as air temperature, incoming radiation, and snow cover and also on the type of vegetation cover (Oke, 1987). The propagation of the temperature from the surface to the interior depends by the thermal properties of the ground, which filter and smooth the temperature signal, recording only the major surface temperature changes (Haeberli, 1990).

The monitoring of the active layer is fundamental in the evaluation of future climatic scenarios, because both water and CO^2 blocked within permafrost can be released. This increases the fluxes of these gasses to the atmosphere, with a feedback effect on the climate system. For these reasons several scientific programs to monitor the active layer and permafrost changes have been established. Within the framework of GCOS such programs are CALM (Circumpolar Active Layer Monitoring) and GTN-P (Global Terrestrial Network- Permafrost). Nine Antarctic sites, all situated in Victoria Land, are at the moment included in the CALM and GTN-P programs. Four of these are managed by the Italian project, three by a New Zealand-US project and 2 by the US).

5.4.2 Site Location

In order to minimize the high variability of active layer thickness related to specific cryogenic features (sorted and unsorted patterned ground, solifluction and gelifluction lobes) that can mask the climatic impacts on the ground, it is really important to locate the monitoring sites in areas without or almost without these features. Sites should also be far enough from large water bodies (lakes) and far from geothermal anomalies (hot springs, active volcanoes) that can influence the thermal regime of the surrounding permafrost and active layer.

To simplify the modelling of the climate impacts on the active layer it is recommended to choose sites with homogeneous topographic characteristics (elevation, slope, aspect, distance from sea shoreline, distance from glacier front) and where possible to use relatively flat (0-5°) sites, in a somewhat higher position that the surrounding areas, and therefore without much local shading effects.

The thermal properties of the ground change with the chemical composition, the grain size and the water content of the deposit. Hence is preferable to use a site with sedimentological, pedological and hydrologic homogeneous conditions. Sampling of the sediments is required. Finally, the sites should be characterised by a homogeneous cover and type of vegetation.

5.4.3 Active Layer Monitoring

The majority of the terrain of the ice-free areas in Antarctica is characterised by coarse sediments (gravel to boulders), making simple frost probing using small-diameter metal rods is not possible. For these reasons it is better in these conditions to use the measurement of the thermal regime of the active layer to monitor

its thickness changes. In the coastal areas of Continental Antarctica and the Sub-Antarctic islands the salt content can be very high, lowering the freezing point of the ground. The 0°C isotherm therefore not always represents the real permafrost table.
We can approach the active layer monitoring by two ways to monitor the thermal regime.

MP: Monitoring Points with all year-round measurements (advanced level, Annex 5)

These year-round temperature measurements have to be carried out with sensors (pt100, pt1000 or thermistors) with a minimum accuracy of 0.2 °C and resolution of 0.1 °C or better. All sensors, except for the one closest to the surface (-2 cm), have to be installed within a thin-walled PVC tube of 2-3 cm diameter, which is sealed at the bottom and closed at the top (leaving only a small hole for the wires of the sensors). The sensor closest to the surface (-2 cm) has to be installed parallel to the surface directly in the ground, roughly 0.5 m away from the tube with the other sensors. To prevent frost heave and possible air circulation within the tube, a cork or wooden slot can be inserted above each sensor. The instantaneous temperature should be recorded with a time interval of 1 hour for the basic and intermediate level and 10 minutes for the advance level.

An Automatic Freezing Front Monitoring System will become available, which simultaneously records changes in electrical resistivity (mainly due tio the ice in the ground) and changes in temperature, at fixed depths, every 5 cm along the vertical.

The metadata of the Monitoring Points should be recorded following the form in Annex 5.

GTPM: Grid Thermal Periodic Monitoring (intermediate level, Annex 6)

This method consists in measuring the temperature of the active layer several times during the summer period. Temperatures are measured at the same depths as in the Monitoring Points, but not deeper than 30 cm, at the nodes of a fixed grid of 100 x 100 m, divided in 10 x 10 m squares.

The temperature measurements have to be carried out with a handheld temperature logger for instantaneous measurements (using sensors with the same technical characteristics as suggested for the year-round measurements; see above).

The measurements can be done in two different ways. The first one is by digging a small square trench of 20-30 cm deep and 40-50 cm wide, and pushing the sensors parallel to the surface into the ground for at least 5-10 cm at the different depths. Wait for the temperature reading to stabilize. The other way is by pushing the sensors directly into the ground perpendicular to the surface, and measuring the temperature at the different depths, again waiting for the readings to stabilise. Alternatively one can drill a series of small holes (2cm in diameter) 5 cm less deep than the required depth for the measurement, and push the sensors into the bottom of the hole for 5 cm until reach the correct depth.

The measurements have to be repeated at least two times during the summer season, if possible at the same dates every year during the period of maximum thawing (end of December to early February) and within 1 m from node of the grid. All measurements should be carried out on e same day or within a few days. In case the surface is covered by snow, the thickness of the snow also has to be measured.

To interpret the results of the thermal monitoring, soil samples have to be collected from the chosen depths in a trench dug close to the MP. Moisture content can be determined gravimetrically, by weighing before and after drying at 105°C for 24 hours. Subsequently these samples can be used to determine the grain size composition and the mineralogical composition.

Furthermore we need some data about the main local climatic elements (air temperature; wind direction and speed; incoming radiation; air humidity; snow thickness; net radiation), preferably from an AWS close to the study site.

5.4.4 Field observations (basic, intermediate and advanced levels)

The two approaches are combined to provide information at different levels of detail:

	Basic Level	Intermediate Level	Advanced Level
Active layer monitoring points	2 Monitoring Points; 4 depths (2, 10, 30, and *x* cm)	3 Monitoring Points; 5 depths (2, 10, 30, *ft* —5, and *ft* + 5 cm)	4 Monitoring Points; 6 depths (2, 10, 20, 30, *ft* —5, and *ft* + 5 cm)
Grid Thermal Periodic Monitoring	No GTPM	GTPM, at 3 depths (2, 10, and 30 cm)	GTPM at 4 depths (2, 10, 20, and 30 cm)
Soil moisture measurements or monitoring	2 soil samples (10, 30 cm)	3 soil samples (10, 30, *ft*-5cm)	4 soil samples (10, 30, *ft*-5, *ft*+5cm) OR Automatic ground moisture content with Vitel or TDR methods *)
Climate data	1 Air Temperature (1.6 m)	1 Air Temperature (1.6 m)	2 Air Temperature (1.6 m, 0.5 m)
	1 Incoming radiation (1.6 m)	1 Incoming radiation (1.6 m)	5 Incoming radiation (1 at 1.6 m, 4 at surface)
		1 snow thickness	4 snow thickness
		1 air humidity (0.5 m)	2 air humidity (0.5 m)
		1 wind speed and direction (1.6 m)	2 wind speed and direction (1.6 and 0.5 m)
			1 netradiometer (1.6 m)
			1 pluviometer **)

Legend : *x* = as deep as possible (maximum 1 m); *ft*-5 = 5cm above the frost table; *ft*+5 = 5 cm below the frost table;

*) Vitel and TDR method have to be recorded at the same depths and with the same time interval of the thermal monitoring.

**) Only in Maritime Antarctic and Sub-Antarctic Islands.

5.4.5 Data processing and archiving

Among the different methods of data processing to obtain the active layer thickness with the MP I propose two methods:

Level 1: Linear interpolation of the Maximum temperature at all the measured depths and calculation of the depth of the intercept of 0°C.

Level 2:Application of the using the Gold and Lachenbruch (1973) equation:

$$Al = \sqrt{\frac{\alpha P}{\pi}} \ln(\frac{A_0}{T_0})$$

Where A_0 and T_0 are respectively the annual amplitude and average of GST (2 cm); P is 1 year and α is the thermal diffusivity computed according the equation

$$\alpha = \pi/P\ (z_2\text{-}z_2/\ln\ (A_1/A_2))^2$$

α can be calculated using the results of the moisture content, the grain size and the petrografical composition of the collected ground samples.

The GTPM data can be treated following the linear interpolation to obtain the depth of 0°C and plotted on the grid to achieve a contouring surface to point out the areal difference.
The MP profile at the same date and hour of the closest node measurement can be used as calibration of the active layer thickness.

The data should be archived according the following proposed format (Table 2), the Insubria University (within the PNRA) can host the data archive center.

Table 2. Table for summarising the data.

Site Name	Code	Method GTPM (1)	Method MP (2)	Minimum Thaw	Maximum Thaw	Mean Thaw	Date of GTPM	MAGST (°C)
Oasi	1	100X100	2	23 (1) 39(2*)	19 (1) 43 (2*)	21 (1) 41 (2*)	19 dec 2005 1 feb 2006	-3.4

In column 3 the size of grid chosen for GTPM has to be included, in column 4 the number of installed MP has to be included; MAGST = Mean Annual GST. * indicates that the calculation of active layer thickness has been computed with method 2 (Gold and Lachembruch, 1973 equation).

5.4.6 References

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5.5 GENERAL SITE DESCRIPTION FOR COMBINED VEGETATION SURVEY / ACTIVE LAYER MONITORING SITES

(Nicoletta Cannone)

In its science plan RiSCC selected a number of core sites along the Antarctic Environmental Gradient (see the Science Plan and the Implementation Plan for a description of this Gradient). In these core sites most of the RiSCC research will be carried out. However, these core sites are only described by their geographic name and position and may cover a large geographical area (e.g. a complete island). The actual research, on vegetation, in lakes, on freeze-thaw processes, etc. will be executed in a smaller sites, being generally one singular habitat. A core site may therefore comprise of more than one study site, depending on the type of studies to be executed.

For each study site a general description should be made. This description should include the exact location of the site and the description of a number of general features, to enable other people picture the site, and to provide comparable information for all sites. If these descriptions are made in a standardized way, it should be possible that participants in RiSCC can select for certain observations or collections necessary for their individual research from the site descriptions the sites that may be useful to them.

For this general site description the general collection / observation form of Ammex 1 can be used. For sites where active layer monitoring is planned, a separate form is added to provide the information pertinent to CALM-IPA active layer monitoring (Annex 2).

6 Biotic parameters

6.1 RiSCC VEGETATION STUDIES - SAMPLING DESIGN AND METHODS

(Niek Gremmen, Nicoletta Cannone)

6.1.1 Introduction

In this document we will discuss a number of methodological issues relevant to vegetation studies within the framework of the RiSCC program. Although we mainly aim to clarify practical field methods for the collection of data, these methods can not be considered in isolation from other aspects of study design.

Our aim is not to describe as many field methods as possible, but to present a very restricted set of methods which are suitable to answer many of the questions posed in the RiSCC program. We will furthermore restrict ourselves to methods for sampling the biotic component of the ecosystem. We will address some issues related to the sampling for environmental variables, we will not discuss methods for actually sampling and measuring these parameters.

The choice of field methods is influenced by
- the questions to be answered by the study
- other requirements or restrictions, e.g. comparability with other studies
- requirements of the methods for data analysis

Most of what is said here should be obvious, but it has been written down in order to achieve as much standardisation as possible between the RiSCC research projects.

6.1.2 Research questions

The questions to be answered, and the hypotheses to be tested, determine the variables to be measured, and the scale of the study. The key questions posed in the RiSCC program for which vegetation studies are important in providing an answer are especially key questions 1 and 2 (See the RiSCC Science Plan)

In general these questions concern
- relationships between environmental variables (e.g. climate) and biotic characteristics
- at the commmunity level (e.g. species richness)
- at the species level (e.g. aspects of species performance, such as productivity, phenology, reproductive success, dispersal, or simply presence)
- interactions between one species or species group and other species in the community, e.g. the impact of introduced species on species richness
- changes in community composition or species performance over time

The final study design and choice of methods depend on the specific questions to be answered and hypotheses to be tested by each separate project. These questions also determine the spatial and temporal scale of the study. They also determine which methods are suitable for data analysis.

Here we present a number of methods which are applicable in answering many different questions, and should be used whenever possible, to assure comparability between as many studies as possible. Also issues of sampling design are discussed.

6.1.3 Variables to be studied

The aims of a study, and the specific questions to be answered determine the variables that have to be studied. The central theme of the RiSCC program is the influence of climate and climate change on the biological components of Antarctic ecosystems. Thus climatic variables are of primary importance in most of the studies within the RiSCC program.

Biotic parameters to be studied are different aspects of biodiversity, and aspects of species performance (e.g. productivity, phenology,

reproduction, or simply presence).

Aspects of species performance, e.g. biomass, phenological stage, number of propagules produced, etc.) can be observed or measured directly. In contrast, diversity is not easily measured or observed directly, and has many aspects. Even if species richness is used as a measure for diversity, it is not immediately clear how this should be defined. Species richness may be expressed as the number of species per unit area. However, species richness may also be interpreted as the number of species occurring in a specific habitat or community. Because in different habitats the relation between species richness and area is not the same, the use of the number of species in a fixed area may yield opposite results with different areas (Figure 6.1).

An approach using functional group diversity will allow a more direct understanding of the relationship between biodiversity and ecosystem functioning, as well as make comparisons between regions with different biota more easily interpretable. Many authors have used a relatively simple system of growth-forms or life-forms as a substitute for functional groups. In studies involving large variation in climate zones this may be satisfactory, but because of the relatively small variation in growth-forms in the flora of the regions of the AEG, a more sensitive system of functional groups seems to be called for. In the RiSCC implementation plan growth-form and reproductive modes are given as traits used in defining functional groups. We suggest that characteristics related to productivity and dispersal should also be considered. Extensive discussions are available in the literature (e.g. the special issues of the *Journal of Vegetation Science* edited by Lavorel & Cramer, 1999 and Woodward & Cramer, 1996) on functional group classifications and the methods to arrive at such a classification. The design of a general functional group classification for use within the RiSCC program should have considerable priority, and needs careful thought. However, this is outside the scope of the present document.

We suggest that the best option in general is to collect information about the vegetation on a species basis. Species data can later be converted into data on different aspects of biodiversity, and at the same time allow for comparisons of species performance. I expect that species data can also be converted into functional group data. For specific research questions it may be sufficient to collect data on a more aggregated or more restricted level, e.g. on the total ground cover of vascular plants, or the abundance or phenology of a single species.

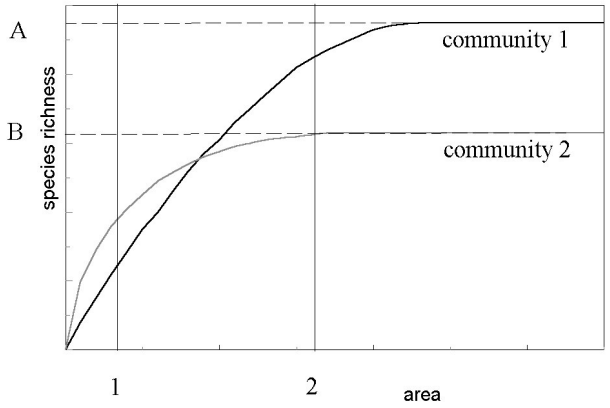

FIGURE 6.1. *Species area curves of two hypothetical stands of two different communities. A comparison of species richness for area size 1 shows community 2 to be richer; a comparison at area 2 shows community 1 to have the most species.*

6.1.4 Data analysis

We do not intend to write a manual on data analysis here. Sufficient easily accessible literature exists (e.g. Sokal & Rolff, Biometry, 1981; Jongman et al, Data analysis in community and landscape ecology, 1997; ter Braak 1996; ter Braak & Smilauer 1998). However, the choice of methods for data analysis has consequences for the sampling design.

Although a very large variety of methods for the analysis of biological data are available, we expect that analysis of variance and regression techniques will be very suitable and commonly used. For community data, obviously multivariate methods are needed. More specialised methods exists, e.g. for time series analysis, but we assume that these will not be routinely used. But in general we suggest that the sampling design should at least allow for the use of ANOVA and regression methods.

Most formal methods for data analysis require independence of observations. Often used methods, e.g. analysis of variance and linear regression also require homoscedasticity (homogeneity of variances in the error terms), and normality of the error terms. Little can be done in the study design to prevent heteroscedasticity and non-normality in the data, and these aspects should be considered in the data analysis stage of the study. If necessary, they can often be remedied by the use of a suitable transformation of the data and suitable methods for data analysis.

Results will be more meaningful and better interpretable when
- there is a good distribution along the range of values of the independent variable(s), and
- explanatory variables are not confounded

A poor distribution along the range of possible values for the explanatory variable(s) may result in observations with high leverage, which may in turn cause erroneous results when analysing the data.

Confounding between explanatory variables (or a high correlation between a measured explanatory variable and one or more factors that have not been considered in the study) may make it impossible to distinguish between the effects of different variables, or even lead to completely erroneous conclusions. In the design of the sampling, it is essential to make sure that correlation between explanatory variables is avoided as much as possible, and that no explanatory variables are highly correlated with other possibly influential variables that are not explicitly considered in the study.

Although informal methods of data analysis do not formally require independence of observations or a representative sampling design, this will strongly increase the validity and interpretability of the results. So also when no statistical methods are used to analyse the data, we strongly recommend adhering to the requirements for representativity and independence of observations.

6.1.5 Sampling design

Issues related to sampling design are e.g. the spatial distribution of sample sites, the number of samples required, the size of the samples, and, for monitoring projects, the frequency of observations. A proper sampling design makes sure that the right kind and the right amount of data will be collected.

Spatial distribution of sample plots

Scale is connected with
- generalisability of the results, which has consequences for the extent of the study area
- spatial pattern in the studied variables, which has consequences for the distance between sampling units, and
- spatial pattern caused by the size of the elements to be sampled (e.g. plants) and patch size of microhabitats. This has consequences for the size of the sampling unit.

The extent of the study area follows from the definition of the aims of the study, and needs no further discussion.

Distance between sampling units
The important measure here is not primarily physical distance, but environmental

difference. Physical distance is only used as an easily measured substitute.
Most formal methods of data analysis require experimental or observational units to be independent. When observations are not independent, the resulting data are autocorrelated. This means that the error terms are correlated across observations that are close in space or time. Autocorrelation reduces the effective number of observations, and thus the power of significance tests. To get equally reliable conclusions from autocorrelated data, even with low levels of autocorrelation considerably more observations are needed. In some cases correction for autocorrelation in the data is possible, but often it is very difficult. The most efficient strategy is to avoid collecting autocorrelated data. This can be done by sufficiently spacing sampling units.

In principle the minimal distance between sampling units can be estimated. However, the effort required to collect the necessary data usually makes this not a feasible option. This minimal distance may differ between areas, and possibly also between habitat types. The only quantitative information I have is based on an analysis of spatial autocorrelation in vegetation pH- and moisture indication data from the Netherlands from a project aimed at studying indicator values at a regional level. This suggested a minimal distance between plots of ca 2 km. This is for plots of the same habitat type. Plots of different habitat types may be spaced closer together.

As a general rule, we suggest that sample plots are spaced far apart, and that multiple observations from a single stand or patch of homogeneous vegetation should generally not be considered as independent. Absolute distance is obviously related to the scale of the study. In a broad-scale study, covering e.g. King George Island, plots should be spaced much further apart in an absolute sense than in a study of fine scale patterns in moss banks on Signy. Lacking quantitative data, the researcher should use his/her own judgement.

Location of sample sites: transects

Sample sites should be spread over the study area in such a way as to yield a representative set of data. A stratified random procedure for site selection generally is effective and efficient.
A special case is the study of changes along gradients. Situating a series of sample sites along an environmental gradient ensures a good representation of the whole range of environmental conditions in the data. One should realise, however, that often along a gradient patterns in species occurrence and abundance occur which are caused by other factors than those explicitly studied. Examples are the occurrence of species in patches caused by vegetative spread, or patchiness in the occurrence of species caused by the fact that propagules predominantly disperse over very small distances. As a result plots close together will be more similar than plots at greater distances from each other (see the discussion on spatial autocorrelation in the previous section). Therefore plots should not be located closely together, and care should be taken to ensure that the spatial extent of the gradient under study greatly exceeds the size of this patchiness in the vegetation.
Otherwise we not only violate the requirement of independence of the sampling units, we may also introduce seemingly systematic patterns in our data which are actually caused by processes which are not included in our study. Thus, when sampling along a gradient, care should be taken that the physical length of the transect, and the distance between sample locations within the transect, is much larger than the size of the patterns caused by the above dispersal processes. Obviously, when these dispersal processes or other fine-scale patterns are the subject of our study, the dimension of scale in our study changes.

A contiguous series of sample plots along an environmental gradient may serve to illustrate and document the pattern in species presence or abundance visually, e.g. in a graph or a map. However when a formal analysis is needed of the relationship between the occurrence of the species, or some other characteristic of the vegetation, and the value of the environmental variable (maybe even simply the position along the gradient), spatial autocorrelation may greatly reduce the effective number of observations, and thus the power of the analysis. An autocorrelation of 0.3 reduces the effective number of observations by 50%. This means that double the number of sample plots are needed to get the same power in the analysis compared to independent data. Once

the data are collected, the spatial autocorrelation can be estimated, and the statistical tests adapted accordingly by reducing the number of degrees of freedom. However, it is much more effective to avoid spatial autocorrelation in the data. Rather than analyzing 100 contiguous plots along the gradient, a much smaller number of well spaced plots should be studied along transects in a number of different sites.

Size of sample plots

In vegetation studies we want to sample in a single observational unit patters of a sometimes large number of biotic variables (species or species groups) of different sizes and spatial distributions. This would suggest the use of large sample plots. At the same time we want the sample unit to be representative for a narrow range of values for one or more environmental variables. This often suggests the use of small sample plots, to minimize internal variation. Large plots take more time to study than small plots, and this is also a consideration in deciding on the optimal plot size.

Despite serious theoretical discussions, the concept of minimum area is useful in determining an efficient sample plot size for vegetation sampling. With increasing plot size within a homogeneous stand of vegetation, the number of species present first increases rapidly, but then tends to level off. Samples of the size where the species-area curve levels off are considered representative for the vegetation in the homogeneous patch sampled. This minimum area differs for different communities, and is related to size and patchiness of the species in the vegetation, as well as to the spatial distribution and patch size of microhabitats. Plot size not only affects the number of species within the plot, it also affects the variance in estimates of the cover value of the species. To reduce the variance in cover values, plots should be considerably larger than the patch size of the species in the vegetation. Data for the determination of the minimal area are usually collected from a series of nested plots of increasing size, each plot being twice the surface area of the previous one. For each plot a full species list is made. Species numbers per plot are then graphed against surface area. A description of the method is found in e.g. Mueller-Dombois & Ellenberg (1974).

Minimum area estimates for Antarctic vegetation range from 50 x 50 cm for Antarctic lichen communities, or even smaller for some bryophyte communities, to 10 x 10 m for some Sub-Antarctic feldmark vegetation.

If the purpose of the sampling is to get representative information on the homogeneous patch of vegetation, which is being sampled, as is generally the case in phytosociological studies, sample plot sizes often differ between habitat or community types.

Because species number is related to area, plot size is an important aspect to consider. Given the relationship between species number and plot size (figure 6.1) and between variance in species cover and plot size (figure 6.2), it is clear that small plots may underestimate the species richness of a community, while also causing large variation in cover data. These same graphs show that an increase of the plot size above the minimum area does not greatly alter the species number nor the cover estimates. Table 6.1 gives suggested plot sizes for different community types. In specific cases there may be a good reason to use other dimensions.

In permanent plots different dimensions may be used, because it is impossible to analyse a large plot without trampling through it, and thus influencing the vegetation. When a lot of natural trampling occurs, e.g. in seal or penguin colonies, a little bit of human trampling will not significantly affect the vegetation, but in bryophyte communities on wet substratum, the impact of even one person trampling through the sample plot may be disastrous. The results of several smaller plots can be added to obtain a realistic estimate of diversity and cover.

The number of observations / replicates

Given a detailed quantified question, it is possible to determine how many data points are necessary to answer this question with the required confidence level and power, using a set of preliminary data. At the moment we have insufficient data, and insufficient

quantification of the research questions, to make even a rough estimate of the number of plots required to answer the type of questions posed in the RiSCC program. This issue will have to be addressed once concrete projects have been started.

Temporal aspects

Aspects of temporal scale will be discussed in the section on vegetation monitoring. However, also in the study of spatial patterns, some consideration needs to be given to the temporal aspect.

Vegetation changes in the course of the seasons, both in species composition by the development and dying of annual species, and in species cover by seasonal growth and die-back. These seasonal fluctuations will be larger in some communities than in others.

To make data from different sites, areas, and times comparable, they all have to be collected during the part of the year when the vegetation is fully developed (except of course when seasonal variation is explicitly included in the study). Special care has to be taken not to confound seasonal differences with other variables (e.g. in a comparative study between two areas by studying one area at the beginning of the season, and the second area at the end), without good reason to assume that the impact of seasonal differences is negligible.

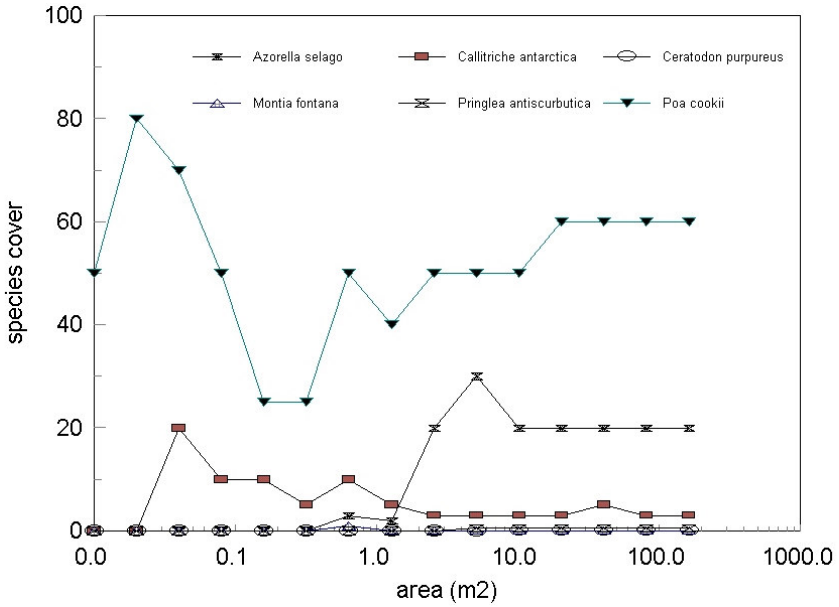

FIGURE 6.2. *Species cover at different plot sizes in Poa cookii tussoch vegetation at Heard Island (Gremmen, unpublished data)*

TABLE 6.1. *Suggested standard plot sizes for different Antarctic plant communities*

homogeneous lichen communities	0.5 x 0.5 m
bryophyte-dominated communities (moss-banks, mire, bog, flush)	2 x 2 m
short tussock grassland and meadow communities	2 x 2 m
herbfield	3 x 3 m
fernbrake	3 x 3 m
tall tussock grassland and megaherbs	5 x 5 m
feldmark	5 x 5 m

6.1.6 Field methods

Plot location in the field.

Sample plots are located within homogeneous patches of vegetation. A homogeneous patch of vegetation is an area within which there are no obvious trends in species distribution. Formal methods are available to test for homogeneity. The work involved usually precludes their use, except when homogeneity is explicitly studied. Usually homogeneity is assessed visually: if the patch is divided in four quarters, do all species occur in each part equally? Also if the patch is divided into three zones in one direction, and also in a direction perpendicular to this? If no patters in species distribution and vegetation structure are seen, the patch is, for practical purposes, considered to be homogeneous. If a clear zonation or some other large-scale pattern is observed, this may indicate an environmental pattern which may have to be considered explicitly when selecting sampling sites.

The exact location of the sample plot within the patch is not of great importance, but observer bias in selecting the location should be avoided. This can be done by some formal procedure for choosing a random location within the patch, but simply throwing a stick or some other object without aiming for any specific spot, and placing the plot where the stick has landed will give satisfactory results. Once the plot location has been determined, a final check for homogeneity of the plot should be made, in a similar way as the check on the homogeneity of the vegetation patch. If the plot turns out to be inhomogeneous, the possible cause should be assessed: is the difference between the different parts of the plot caused by some environmental pattern? Or is it caused by characteristics of the species (e.g. a large clonal patch of one species covering one half of the sample plot). In the first case a new location for the sample plot should be chosen. In the second case maybe the plot size is too small in relation to the size of the plant units.

Making species lists

In some studies only a limited number of species will be studied, or other aspects of the vegetation, like the cover of different life-forms, or the vegetation structure. Generally, however, comprehensive species lists will be made. Since the observers are human, there is plenty of room for observer differences (bias). Therefore it is important to standardise procedures as much as possible.

This is done by first listing all species which are immediately visible to the observer. Once this is done, the sample plot is searched systematically from one end to the other and every species encountered is listed. Seedlings are listed separately from adult plants. Care is taken not to disturb the vegetation more than necessary, so as not to influence the species cover.

In layered vegetation, e.g. consisting of a herb layer and an understory of small herbs, bryophytes and lichens, the species are listed per layer.

Once all species have been listed the cover of each species is estimated. See the relevant section for the best way to do this.

Species that cannot be identified with confidence in the field have to be collected for later identification. Make sure to keep a good administration of what was collected where. Write the number of the collection down with the sample plot data, and write the identification of the sample plot on the specimen label.

When plots are used for repeated observations, e.g. in a monitoring study, any impact on the vegetation by the observer should be avoided. The field-worker should stay outside the sample plot, and not remove plants (or take soil samples etc.) from the vegetation sampling plot. When species need to be collected for identification, specimens should be taken from the area outside the plot. Also soil samples etc. should be taken from the area immediately outside the vegetation plot. See the section on vegetation monitoring.

Often it is not easy to know when all species have been recorded. In sample plots in open, very species poor vascular vegetation, this will be simple, but in dense, relatively species rich vegetation this is not so easy. Especially tiny bryophytes which occur in very small numbers in between other species make it difficult to

decide when all species really have been noted. In general my procedure is the following: the whole sample plot is searched systematically from one end to the other, in strips of ca 20 cm wide. If in this search a new species was added to the list, another complete search is made. Once a complete systematic search of the sample plot does not yield any new species, it is assumed that the species listed are a good approximation of the species composition of the vegetation. The time needed for analysing the vegetation of a sample plot depends on the structure and species richness of the vegetation. In very species poor vascular plant communities 15 minutes or less suffice. In structurally complex, species rich bryophyte and lichen communities, it may take up to several hours. Obviously this also depends on the size of the sample plot.

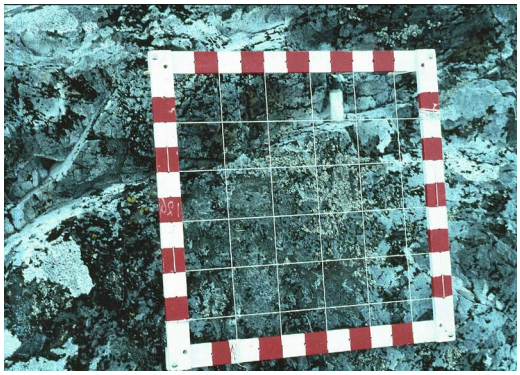

FIGURE 6.3. *Sample frame*

Estimating plant cover

Within sample plots, plant cover can be estimated or measured. The most widely used method is to visually estimate the cover of each species in the plot. The observer first lists all species that occur in the plot. Subsequently for each species the cover percentage is estimated. Advantage is that it is quickly done. Drawback is that this method is not necessarily very accurate, and may be influenced by observer bias. The use of a frame which can be placed over the sample plot in the field, marked with e.g. 10 cm divisions, and if possible with a 10 x 10 cm string grid (elastic works well, cf. Fig 6.1) will appreciably reduce observer error and bias in estimating species cover, and is highly recommended. By simply counting how many 10 x 10 cm squares are covered, adding partly covered squares together, the total cover of a species can be estimated quite accurately. In the Maritime and Continental Antarctic a smaller grid may be more applicable, e.g. 50 x 50 cm, with divisions of 5 cm.

In many studies a cover abundance scale, like the Braun-Blanquet scale is used, in which cover is estimated in a limited number of (unequal) classes. For general purposes it is better to estimate the cover in straight percentages. These can always be reduced to classes, while the reverse is not possible. Below 1% cover one should record the number of individuals, in 4 classes: 1-3, a few, many, very many (comparable to the classes r, +, 1 and 2m on the extended Braun-Blanquet scale, see e.g. Westhoff & van der Maarel, 1973).

An alternative is the use of a point-intercept method to estimate plant cover. Here the sample plot is also divided by a grid, and at each intersection of grid lines a pin is lowered vertically into the vegetation. Each species touched by the pin on its way down towards the substratum is noted. From the number of hits per species the species cover can be calculated. Advantage is that, if executed correctly, this method leaves no space for observer bias. If a sufficient number of points are used, cover may be estimated quite accurately. Rare species may be missed, unless a very dense grid of points is used, and thus species richness will often be underestimated. Another drawback is that this method is very time-consuming. In using a point-intercept

method it is vital that the pin diameter is standardised. Differences in pin diameter result in appreciable systematic differences in estimated cover percentage (see e.g. Table 1 in Shimwell 1971).

A third approach is to count the number of individuals in the sample plot. In very open vegetation, with individuals of roughly the same size, and when studying colonisation processes, this may be a suitable method. It is not suitable for general use in the RiSCC program, however.

We suggest that a visual listing of all species and a visual estimate of the cover of all species is the standard way to collect data on species composition and species richness of the vegetation. We suggest using a frame, and if at all possible a grid to aid in estimating the cover percentages.

Some authors advocate the use of a number of small-sized subplots per sample site. They for instance make species lists and estimate or measure species cover in 10 1-m2 subplots within a 100 m2 sample plot. The implied rationale is that these replicate subplots allow for a better estimate of the sample value (the mean of the subplots) than one single 10 m2 sample plot, and thus reduce the random variance in the data. When a contiguous series of subplots is used, I see no reason to expect better data than from a single larger plot. In case the subplots are spread over a larger area (e.g. 10 1 m2 plots randomly distributed within a 10 x 10 m sample area), the variance may be somewhat smaller compared to a single 10 m2 sample plot, but it is not clear if this is worth the considerable extra effort. No data to test this are available.
In the analysis of the data, sub-plots should not be treated as separate observations (pseudoreplication). This obviously violates the requirement of independence of the data points, and will lead to invalid results.

6.1.7 Vegetation monitoring

Monitoring to detect invasions or to detect changes in species distributions is not treated here. If this is necessary, we can write a separate document.

The aim of vegetation monitoring is to detect changes over time. Generally we also want to relate these changes to changes in other (environmental) variables. In the RiSCC program climate change is of primary importance.

Two aspects are of interest
- technical aspects of vegetation monitoring
- how can we relate vegetation changes to changes in other variables, especially climate?

To start with the last:
We again need a study design in which we can distinguish changes caused by climate change from changes caused by other factors. This requires a study design in which climate change is not correlated with changes in other environmental variables. Because we can exert very little control over climate change in observational studies (it can be done to a limited extent in an experimental set-up), this will be extremely difficult. What is needed is a set of sites which have different amounts of climate change, but which are otherwise completely comparable.

The methods for vegetation analysis are in principle identical to those in other vegetation studies.

Vegetation monitoring often uses permanent sample plots. One requirement is that the exact position of all plots can be found again for the next series of observations. This requires the use of field markers. If a plot can not be exactly located again, two courses of action are possible: 1) the best approximation of the original position is used, or 2) the time series is stopped, and a new plot is established, and a new time series started. In both cases there is a break in the series of observations, which introduces variation which was not introduced in the other series of the study. One solution is to start with a larger number of permanent plots than the study requires: if some plots are lost (as some are bound to be, for many different reasons), this will not deleteriously affect the study.

Another solution is not to use fixed position sample plots. Instead relatively large, homogeneous study sites are used, within which one or more randomly located subplots are analysed. Advantage is that it is much less

likely that the large study sites will not be found again for the next series of observations, and thus no extra variance is introduced by breaks in the time series. Also as a part of the study site becomes unsuited for further study (e.g. by a bird digging a nest in the middle of it), this part can be avoided in subsequent sampling without significantly affecting the study. If this bird had built its nest in the middle of our 1-m2 permanent quadrat, this would have meant the loss of the site. Temporal autocorrelation in the data may be expected to be smaller when using variable plot locations rather than fixed quadrats, and thus a smaller number of observations may be necessary to reach the necessary number of effective observations. Drawback is that extra variance is introduced by analysing a different plot each time. This may be countered by analysing a number of plots at each study site, and pooling the data per site (extra effort). We presently have no data to quantify these aspects of the sampling design.

Fixed sample plots are generally used in vegetation monitoring, but they have serious drawbacks. The most important is the possibly very high temporal autocorrelation in the data from a fixed plot. This may vastly reduce the effectivity of the sampling, and result in the need for considerably more plots to reach the required reliability of the results. Another drawback is that often the precise location of plots is impossible to find again, resulting in breaks in the time-series data.

If the period available for the study is long, e.g. several decades, temporal autocorrelation in the data can be reduced simply by spacing out the observations in time, e.g. by a frequency of observation of once every five year. However, the time-horizon of the RiSCC program is only ca 7 year. This means that changes have to be detected rather quickly. Two approaches can be used to counter the reduction in the number of effective observations due to temporal autocorrelation in the data. One is simply to double or triple the number of permanent plots (we do not have sufficient data to make a good estimate of the necessary plot number). The second approach is to minimise temporal autocorrelation by not using fixed sample plots, but variable position plots, e.g. by using random plots within larger study sites. Without quantitative data it is difficult to decide which course to take. When temporal autocorrelation is low and high between-subplot variance fixed plots are preferable. However, when temporal autocorrelation is high, and between-subplot variance is low, the use of random subplots within study sites is definitely preferable.

Another consideration when designing a monitoring study is the frequency of observations / measurements. Obviously this frequency depends to some degree on the aims of the study. When differences between seasons are of interest, observations will be made several times a year. However, when the main interest lies in the effects of climate change, one does not expect to detect these effects within a few months or even years. So we need long-term monitoring, say between 10 and 100 years. It is somewhat unrealistic to start planning for a 100 years time series study, but on the other hand, we should not preclude the possibility. This means that any monitoring study should be designed in such a way that it can be continued later, e.g. that the sites can be found again and are kept intact. Do not after 5 years go and remove all field markers, trampling through the plots in the process. It requires usually little effort to leave proper markers in the field, and to take some care that sample plots are not destroyed, so when the need arises, the monitoring network can be revived again with minimal effort, and minimal introduction of extra variance.

Although the effect of changes in climatic variables in some cases may be more or less instantaneous (e.g. the effect of ambient temperture changes on photosynthesis), many effects will take longer, and some may even have large lag times, or result from some kind of integration of climate over a long period (e.g. changes in species biogeographical areas, related to length of growing season). Aspects of vegetation that will be studied in the RiSCC program will be

- species composition
- species richness or other diversity measures
- vegetation structure (biomass, cover, height)
- species performance
    - abundance
    - phenology
    - reproductive success

Different aspects require different time scales,

and a different frequency of observation. Species composition and species richness usually show little short-term fluctuation (if we exclude annual species), and are typically studied over long time-scales with a low frequency of observation. Biomass, plant height, and phenological stage may change rapidly over the course of a growing season, and require more frequent observations. However, we may not be interested in e.g. the changes in biomass during the year, but e.g. in a possible trend in the maximum biomass of a set of species over a long period. In that case frequency of observation may be low.

This brings us to the point of cyclical changes with a period longer than the length of any single observation, but much shorter than the total time scale of the study. An obvious example is seasonal variation. In many studies this seasonal variation will not be of interest. It is important to reduce variation due to seasonal fluctuations as much as possible in the data, e.g. by always sampling at the end of the growing season, and to avoid any confounding of the different sources of variation. For instance when testing for differences between a set of data from year A and year B, sampling in year A should not be done in November and in year B in February, unless it can be shown that seasonal differences are negligible, or unless one can correct for the effect of season. This would require a suitable set of data on seasonal variation.

Again observations should be independent, i.e. in the analysis of the relationship between the studied variables the errors (residuals) should be randomly distributed. Residuals of successive observations should not be more similar than of observations at very different times. Temporal autocorrelation in monitoring data will result in overestimating the significance of differences or trends, overestimating the power of the monitoring system, and results in an inefficient use of resources, because the effective number of observations is reduced. I do not have data for the Antarctic, but in vegetation monitoring data from The Netherlands a temporal autocorrelation of ca 0.3 was estimated for observations from subsequent years. Monitoring with a frequency of once every year reduced the effective number of observations by ca 50%, necessitating twice as many sample plots to get the same conclusions as when the data would be uncorrelated. (I have some data from Marion Island, and will try to analyse them one of these days).

In general for monitoring I would recommend relatively small plots, 50 x 50 cm for lichen communities, and 1 x 1 m for all others. Only in large tussock grassland 2 x 2 m would be preferable. The main reason for small plots is that it is possible to study them in detail without disturbing them. When deemed necessary, one can use several 1 m2 plots close together, but with enough space between them to stand while making the observations, to make up one sample. This could be done e.g. in feldmark, and large tussock and megaherb communities.

Another issue is the use of data from previous observations when making observations. Sometimes people will use species lists from previous observations to check if all species have been recorded again. Obviously this may reduce observer error in the repeat-observation, but at the same time it will introduce systematic differences. For the first observation of the time series no previous data were available, and observational error may be expected to be larger here than in repeat observations. And a systematic difference will be introduced because in a repeat-observation one will search specifically for all species previously listed. As a result there may be expected to be less errors in the detection of disappearances than in the detection of new species in the plot. To prevent this kind of bias, all observations should be made without reference to the results of earlier observations.

Annex 3 provides a form for vegetation survey data.

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6.2 PHENOLOGY / REPRODUCTION

(Dana Bergstrom, Yves Frenot, Françoise Hennion)

The objective of this type of study is to compare the phenology of different species species (e.g. *Pringlea antiscorbutica, Acaena magellanica, Poa cookii, Azorella selago*), between different sites along the AEG, in different habitats, or along altitudinal gradients. In studies of the phenology of the species, different methods of data collection are used. Generally used classes to indicate the phenological stage are:

seedling
juvenile plant
vegetative, adult plant
plant with flower buds
flowering
fruiting, fruit not ripe
ripe fruit, fruits dispersing
dead

for bryophytes:

gametangia present,
fresh spore capsules present,
empty spore capsules present

for lichens: apothecia

present,
soralia and/or
soredia present.

Often the phenological stage is routinely noted. The effort is negligible, and it may contribute to a set of useful data. When not all plants of a species are at the same stage, this may be noted, e.g 10% flowering, 90% vegetative. For more precise studies the following protocol can be used:

Site establishment

Establish sites in the appropriate habitat types. When you are interested in the effect of climate differences, it is important to select similar habitats (moisture, nutrient status, substrate characteristics) at each site. Record the full set of RiSCC site data for each site (see Annex 1 and 3). If at all possible, collect climate data using an AWS for each site, or a sensible selection of sites.

Choose 20 plants within each site which are typical of the site within an area of around 30 x 30 m to 50 x 50 m. Tag or flag the plants:

Pringlea antiscorbutica – mark the plants with a loose cable tie with a tag below the leaves, and flag each plant, e.g. using a plastic stick with label.

Azorella - select either an entire cushion (if the cushion is smaller than 30 x 30 cm) or mark out a 30 cm x 30 cm quadrat on a cushion, using green puffy paint and pins or plastic sticks with a label.

Acaena - select and tag (with cable ties) 10 inflorescences within a mat of *Acaena*. Alternatively mark out a 20 x 20 cm quadrat in the *Acaena* mat, using two plastic sticks in oppposite corners of the quadrat, and use all inflorescences within this quadrat for the phenology study (Figure 6.4). Flag the plants or quadrat, e.g. with a plastic stick with label.

Poa cookii - tag 5 inflorescences per plant with cable ties and mark inflorescence leaves with puffy paint. Flag the plant.

Plant observations

At each site, at each visit measure % of total for each phenology stage on the 20 tagged plants or sample quadrats. Visits are repeated according to a fixed schedule, which should be the same for each site. Given the short length of the season in many parts of the AEG, repeat visits every 5-7 days are necessary for a precise picture of the phenological development of the plants. A form is provided in Annex 7.

The following seven phenological stages are discerned for *Acaena magellanica* (once buds are visible). For other species a similar set of stages can be defined. Each stage is very easy to identify in the field (using a magnifying glass at the beginning, then it becomes obvious):

1. Buds
2. Stigma erect, anthers not ripe and short
3. Stigmas dry / flaccid, anthers fat or opened
4. Anthers dry
5. Spikes emergent
6. Spikes well developed
7. Fruit ripe and breaking up

(at this point, collect the seeds)

Plant reproductive capacity

The reproductive capacity of the species can be assessed in the same sites or along the same altitudinal transects as the phenology. Data are collected on the last phenology observation date, except when fruits are ripe at an earlier date and are already shedding.

Pringlea antiscorbutica.

Use 15 plants randomly chosen among the 20 *Pringlea* phenology plants. The following is measured:

- On each plant: count the total number and measure the length (cm) of each inflorescence, measuring from the lowest silique to the top of the inflorescence; count the number of inflorescences eaten by rodents. Note these in table.
- On the MAXIMUM length inflorescence (or one of the MAXI), count the siliques;
- On this MAXI inflorescence, randomly sample 15 siliques and bag each silique individually (in small paper bags).
- Randomly choose another inflorescence, if any exist; repeat point 2 & 3 - count the siliques; random sample 15 siliques, bag each silique individually (in small paper bags).
- From each collected silique, count the number of fully grown seeds and aborted seeds. Keep the seeds per group of 15 siliques.

Azorella selago.

Use 15 of the *Azorella* phenology plants. The following is measured:

- Count the total number of flowers. If the number is too high, count only on a sector (1/4 of the cushion for example) and deduce the total number from this counting.
- Pick 10 flowers from the cushion and bag each individually (small paper bags) following the pattern below.
- From each collected, count the number of fully growing seeds and aborted seeds.

Acaena magellanica

Use the 15 *Acaena* phenology plants/quadrats. The following data are collected:

- Collect the 10 marked inflorescences
- From each collected inflorescence, count the number of fruits and divide into fully growing fruits and aborted fruits. Air dry the fruits.

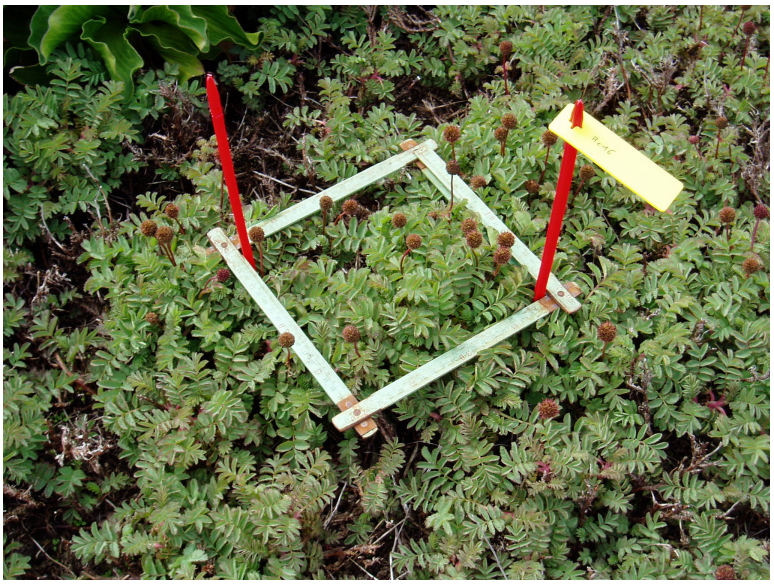


FIGURE 6.4. *Acaena marked in the field for phenological studies. The two sticks mark the sub-plot, while the square is only used during observations.*

6.3 MORPHOMETRY

(Dana Bergstrom, Yves Frenot, Françoise Hennion)

To study the relationship between environmental conditions and plant morphology, samples for morpholmetrics are taken using a similar observational design as for the phenoloy studies. Sampling takes place in selected habitats along altitudinal (as a proxy for climate) gradients and/or along latitudinal gradients. Habitats are characterised by nutrient status, moisture and exposure, and care should be taken to avoid confounding between habitat characteristics and latitude and/or latitude. The following protocol was used in the RiSCC-4-Islands study.

Two types of data are collected from the leaves: morphometrics and specific leaf area (surface area/ dry weight).

Sampling protocol

The aim is to collect at a minimum for each key area plants from five altitude levels. At each altitude collect:

5 plants from very exposed sites
5 plants from very protected sites
5 plants from intermediate sites

Exposure: use the 5-point wind exposure scale as defined by Bergstrom & Selkirk (2000) to describe the degree to which a plant (or site) is exposed to the wind. For this collection we use only three of the above categories:

- A *very exposed* site is one that is wind blown from many directions. There may be ventifacts.
- A *very protected* site is one that is very sheltered from the wind by landscape features.
- An *intermediate* is the middle of the extremes

For each species, individual plants should be as far apart as possible (minimum 50m) to avoid having to take into account issues of spatial autocorrelation.

Site description

Each plant will be considered to be an individual site, Thus leaf collections from each plant will need site data collected. For each site (i.e. plant), a single soil sample will be collected. Take 1 soil sample per plant within the rhizosphere, for soil moisture content, pH and electrical conductivity. Measure depth of soil (unconsolidated substrate depth, USD) using a probe to 1 m.

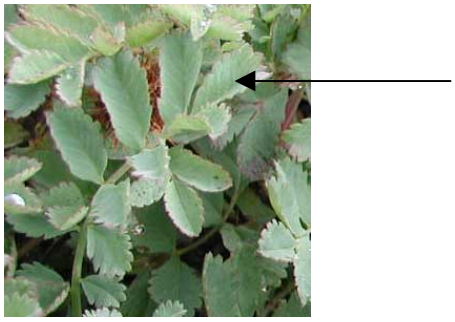
Plant collection

When selecting the plant (site), randomly select a mature healthy plant, *relative to the degree of exposure*. Measure the height and diameter of each plant (with some exceptions – see table below).

Collect the youngest fully expanded leaf or leaflet (for *Acaena* see photo below). Place leaves into separate envelopes, and place envelopes in a zip-lock plastic bag to reduce water loss before scanning. DOUBLE LABEL EACH BAG with a sticky paper label on outside and a plastic label inside. For convenience, clip envelopes together with strong turnback clips. This will keep envelopes closed but not sealed, and will allow us to re-use the envelopes.

Azorella: collect leaves from a top branch of the cushion
Acaena: collect only the apical leaflef
Grasses: collect leaf blade + sheath.

the arrow shows the apical leaflet of *Acaena* to collect for morphometric studies



Back at the base or hut, measure fresh weight of leaves before scanning

Scan leaves using scanner. Make sure that there is a measure of known surface area on the scan for calibration. *Grasses*: When scanning the leaf, mark the border between leaf and blade

Drying. After scanning, place leaves flat into small paper bags – dry by either microwave or air-drying. Microwave all *Pringlea* leaves to prevent fungal attack while drying (air-dry the rest). *Grasses*: Cut sheath from leaf and determine dry weight of each separately.

Dry weight of leaves and soil (see below for soil notes). The dry weight will be measured in the lab, after re-drying leaves and soils in an oven:

Leaves : 65 °C until a constant weight is obtained

Soils : 105 °C until a constant weight is obtained

Physiological and molecular level

6.3.1 (Eco)physiological studies on the RiSCC key species

(advanced level)

Within the context of RiSCC studies in the following fields may be planned:

Microorganisms
- Photosynthetic, chemosynthetic and respiration rates
- Uptake, assimilation and aloocation
- Temperature and desiccation tolerance
- Growth rates

Macroscopic algae (Prasiola crispa)
- Photosynthetic and respiration rates,
- photosynthetic efficiency,
- water balance,
- growth rates,
- form variation

Mosses and lichens (Usnea, Umbilicaria, Ceratodon, Polytrichastrum alpinum, Sanionia)
- Size, growth form (lichens), shoot density (mosses)
- Photosynthetic and respiration rates
- Water balance
- Reproductive mode, reproductive characteristics
- Spore counts
- Photosynthetic effeiciency
- Chlorophyll content (lichens)

Vascular plants
- Photosynthetic and respiration rates
- Size
- Water use efficiency
- Inflorescence number, seed weight, seed number
- Specific leaf area
- Stomatal density
- Height of plant
- Root system

Photosynthetic efficiency
- Invertebrates
- Size, fluctuating asymmetry
- Respiration rate
- Thermal tolerance
- Egg size and number

At this moment no standard RiSCC protocols are available for these studies.

6.3.2 Sampling for biochemical and genetic studies

(advanced level)
(*Françoise Hennion, Sharon Robinson*)

In some studies it is important to collect samples from many different areas, which can not all be visited by a single person within a single season, or even a few seasons. Therefore it may often be important that third parties are able to take samples for these studies. Samples collected for genetic and biochemical studies often require specific treatment. In the table (below and annex 8) we give general guidlines for the treatment of samples for different studies. For a general description of sample site characteristics the general collection / observation form of annex 1 should be used. In annex 8 a form is given to record details of samples taken for genetic or biochemical studies.

SAMPLE FIXATION/ CONSERVATION GUIDE

Type of analysis		Immediate fixing/ freezing required	Appropriate type of collection						
			Frozen in liquid N2	Frozen (-20°C)	Solvent	Oven dried	Micro wave dried	Silica dried	Lyophilised
DNA damage		Yes	X						
DNA sequencing			X	X			X		
Photosynthetic pigment		Yes	X						X
UV-B absorbing pigments			X	X		X			X
polyols/ HPLC		Yes	X	X					X
sugars/ HPLC		Yes	X	X					X
amino acids/ HPLC		Yes	X	X					X
Polyamines	**Free**	Yes	X	X	Hydrochloric Acid 1N				X
	Conjugated	Yes	X	X	Methanol 100%				
NMR		Yes	X	(X)	Perchloric Acid				X

6.3.3 Collecting leaf samples of Deschampsia antarctica for DNA extraction, using silica gel

(Mark van de Wouw)

For a study of the colonization history and diversity patterns of *Deschampsia antarctica* samples of Deschampsia antarctica need to be collected from a wide variety of locations throughout its distribution range. For the sampling of *D. antarctica* leaves, collaboration is sought with other Antarctic programs to maximise the area covered in this study. The number of samples to be taken is flexible (any sample will greatly help this study), but ideally we would like to have samples of at least one population per location, with 10 plants sampled per population. Because we are interested in the relationship between genetic and physical distance, the distance between sampled plants needs to be recorded. As we are using molecular techniques such as AFLP's and sequencing of chloroplast DNA, a special sampling protocol need to be followed to obtain good quality DNA. This ptotocol is a modification of a technique used for tropical trees, described in Weising et al. (1995).

Sampling protocol:

1. Leaves should be collected from ten plants per population
2. If the size of the population allows it: sample a transect of 100 m, where at intervals of 20 m two plants will be sampled. When the population does not allow sampling of a 100 m transect, the ten plants can be sampled be closer to each other. If the population has less then 10 plants, then each plant should be sampled.
3. For each sampled plant, write down the distances to the other sampled plants and the diameter of the tuft.
4. Harvest the youngest, healthiest leaves you can find (50 mg is plenty). No dirt, no yellow leaves, no roots, no inflorescences (but each sample should be of only one plant!). The number of leaves you need to collect depends on their size. Sometimes, when the leaves are very small, you may need 20 leaves or more, but with large leaves two or three may be sufficient.
5. Place the sample of each plant in a ziplock plastic sample bag (labelled!), filled with dry silica gel
6. Close the bag carefully
7. Change the silica gel every 6 hours (not necessary if a lot of silicagel is used in relation to the amount of leaves harvested)
8. After the second change, the leaves will probably be completely dry. Then put just one third of dry silica gel in the sampling bag for storage. (You can re-use silica gel by re-drying in an oven (at 150°C) or in a pan; make sure it has cooled down completely before you add it to the samples!)
9. Samples can be stored at room temperature, but keep them away from direct sunlight or excessive heat.

At the same time as collecting leaf samples, also collect, for each population, a herbarium specimen (leaves and inflorescences) for future reference and identification. This is especially important when collecting from areas where other grass species occur.

Write down basic information on collecting location:

Collection number and date
Latitude and Longitude
Site description (e.g. place name, distance from other place etc.)
Altitude (meters above sea level)
Slope and Aspect
Exposure (from sheltered to very exposed)
Habitat descriptions (including other plant species)
Size of Deschampsia population (number of plants/extent)
Soil texture
Soil ph (if possible)
Drainage (from dry – waterlogged)
Any other interesting info about the location/population

Reference:

Weising K, Nybom H, Wolff K and Meyer W (1995). *DNA fingerprinting in plants and fungi.* CRC Press, Boca Raton.

NON-MARINE AQUATIC ENVIRONMENTS

7 Investigating Limnetic Systems

(Antonio Quesada)

The presence of free water (at least during summer months) is a feature of all ice free areas of Antarctica and has now been detected in subglacial environments (e.g, flowing water beneath the WAIS ice streams and the numerous subglacial lakes such as Lake Vostok). This water can be in the form of temporary or semi-permanent streams and ponds or lakes with seasonal or permanent ice cover. Many ice-free areas are only infrequently accessed by researchers. Therefore detailed information on limnetic systems is limited to only a few sites, such as Signy Island, the Dry Valleys, and Vestfold Hills, and often to only a few water bodies at these sites. To obtain an accurate picture of the diversity of Antarctic limnetic systems and their communities it is necessary to obtain data from as many systems as possible. It is not essential (nor indeed often practical) to undertake detailed investigations of each and every system but it is particularly valuable to at least identify the existence of a limnetic system and provide a basic description of it. There are three levels of investigation that should be considered.

1. Basic descriptive level - this does not assume any instrumentation being available but equipment such as GPS (or at least a compass) would seem reasonable standard field equipment.

2. Intermediate description level - this would require at least handheld pH, temperature and conductivity sensors and meters.

3. Detailed studies level - this level of study will have its own objectives and could utilize the methodologies outlined in this manual but should ideally also make the measurements outlined under the BASIC level of study.

7.1 AQUATIC NON-MARINE SYSTEMS FOR NON-LIMNOLOGISTS

(basic level; only requires a map, an ice-axe, a pencil and 30 min)
(Antonio Quesada)

These very basic data can be easily obtained, and this information could be extremely useful for global change studies. The data required are listed in the following form (see also Annex 9):

Identifier:		Date/Time:
Observer:		
Location (coordinates)(1) :		
Altitude:		
Name of area and/or water body:		
Type:	Lake/stream/lagoon	
Size:	Diameter	Estimated / measured
	Perimeter	Estimated / measured
Depth:		Estimated / measured
Inflows:	Yes/No	How many?
Outflows	Yes/No	How many?
Ice coverage	permanent	Yes/No
	% coverage (up to): 25, 50, 75, 100%	
	Ice thickness:	
	ice transparency: high / moderate / none	
Water colour:	clear, brown (organic), milky, others	
Zooplankton	Yes/No	
Lake Shores:	Vegetated (up to): 25%, 50%, 75%, 100%	
	cyanobacterial mats (2): Yes/No	
	green algae (3): Yes/No	
	mosses: Yes/No	
	vascular plants: Yes/No	
Catchment	Size (4): 1x size of waterbody, 10x, more	
	Vegetated (up to): 25%, 50%, 75%, 100%	
	Vegetation:moss / lichens / vascular plants	
	Geology (e.g.): volcanic, metamorphic, granitic, sandstone	
	Geomorphology: slope: flat, steep, cliffs	
	Geomorphology: weathering: none, some, extensive	
	Animal influence intensity: negligible / moderate / high / intense	
	Animal influence type: nesting birds / marine mammals	
	Snow and ice (up to): 25%, 50%, 75%, 100%	
	General wetness of the area (polar desert, wet polar area?)	
Other Observations:		

1- give the GPS coordinates of at least one point at the water body or compass bearings to prominent landmarks
2- cyanobacterial mats can be detected by running a finger through the surface of sediments, the appearence would be a different colour than the sediments (brown, orange, blue-green)
3- green algae have the aspect of bright green colour filaments or sheets
4- size of catchment in reference to the waterbody

Sketch of waterbody and ice coverage:

7.2 AQUATIC NON-MARINE SYSTEMS FOR AFICIONADO LIMNOLOGISTS

(intermediate level: requires some instruments, rubber boots or waders, and more time)
(Antonio Quesada)

In addition to the information collected using the form in the previous section, some measurements of environmental parameters (temperature, oxygen content, conductivity and pH of the water), and the collection of some samples (plankton and benthos) will be important.

These measurements, as well as the sampling, can be undertaken from the shore, when open water is available. You do not need a boat for this. If the waterbody is ice-covered, do not proceed onto the ice, unless you are ABSOLUTELY sure that it is strong enough. For sampling properly from the ice you will need drilling gear.

If you are sampling from the shore, the sampling can be undertaken with a bamboo pole and/or a clean rigid tubing with a buoy at the sampling end. Ideally, the sampling point should be over the deep water and not over the shallows, so wade out as far as practical. It is important not to disturb the sediments when sampling. The sample has to be collected from a depth of 20-30 cm below the surface, into a clean plastic water bottle. At any sampling site first wash the bottle and the sampling device with lake water.

Avoid any cross contamination with other water bodies you are sampling by cleaning thoroughly the sampling gear between lakes, pouring the cleaning water out of the waterbody.

The best site to sample a lake from the edge would close to the outflow, since it integrates most of the lake features. If you have the opportunity, take further samples from other representative areas of the lake (inflows, sand banks, etc.). This will provide greater detail on diversity of the system.

Next measurements will be recorded with probes which necessarily should have been calibrated previously (the same day of the sampling). Measurement will be done in the following order:

- temperature and oxygen (usually together in the same probe).
- conductivity
- pH

If you can get a plankton net (50-200 □m) and tow it in the shallows you can have a good idea of the zooplankton as well as the large phytoplankton. For methods to preserve the sample, look at the detailed section later in the manual.

In polar aquatic ecosystems a large part of the activity takes place at the benthos. Therefore a benthos sample should provide useful information. Take a sample from the top 1cm of the bottom sediment or substratum, and preserve it as described in the detailed methods. The sample can subsequently be analysed for flora and fauna (see later sections).

7.3 DETAILED PHYSICAL DESCRIPTION OF THE AQUATIC ECOSYSTEM

(advanced level)
(Enn Kaup, Manuel Toro, Cynan Ellis-Evans)

The physical description of the system to be investigated includes in the first place a description of the catchment area, geology, snow cover (percentage, water reserve), vegetation, animal colonies, human activity. Referring to the waterbody itself it is important to record data about the ice cover: percentage of ice/snow, thickness of ice/snow, albedo, optical properties (light transmission, development of crystals), particles and chemistry of the ice cover (conductivity, nutrients, major ions). Also the water balance for the waterbody represents data of importance: lake level changes, inflow rates/amounts, outflow, evaporation

Bathymetry - this can be assessed when the lake ice is strong enough to walk on safely, but ideally thin enough for holes to be readily made by a bolt chisel or axe and for the lake edge to be clearly distinguishable.

- A line is laid down the centre of the lake on the long axis and its direction ascertained by compass. Further lines separated by 20-30m intervals are then run at right angles to this reference line to either side of the lake.
- Holes are cut (or drilled with a small diameter ice auger) at 10 to 20m intervals along these lines.
- A small weight can be lowered through each hole to the lake bottom on a graduated line, noting the depth at each hole. Alternatively a handheld sonar device can be held at the water surface in each hole. This seems to measure depth more reliably. Ground penetrating radar mounted on a sledge can also be used to measure depth, particularly on larger lakes, avoiding the need to make holes. This, however, is expensive technology.
- The position of the lake edge with respect to each line is noted and further measurements taken at a sufficient number of intervening points to later draw the shoreline. A number of graphing packages (e.g. Surfer) are available that will draw a contour map and work out depth slice volumes and areas very accurately.

7.3.1 Water column structure: Profiles

(Antonio Quesada)

Many waterbodies are stratified. Even in very shallow systems (20-40 cm), well marked stratification can be present. This stratification can be due to different processes, e.g. salt extrusion during ice formation, ice melting, or water intrusion from the watershed. This stratification may be responsible for biological and ecological heterogeneities, which can explain most of the ecosystem functioning. The physical and chemical structure of the water column provides very valuable data that can help to understand the ecosystem. The profiles of these characteristics can easily be made, using multiparametric probes. The most sophisticated systems are autonomous, do not need to be linked to any computer or reader and store the data in the probe itself, after the measuring frequency and conditions have been programmed in. Usually these systems contain barometric depth sensors, oxygen, pH, conductivity, and temperature probes, all within the same instrument and give immediate profiles of all the parameters. Other sensors, e.g. redox potential, turbidity or fluorescence can also be included in the same instrument. The multiparametric probes are most useful in deep systems (over 2 m) since the length of the probe is considerable and can only give one or few depths in shallow systems. Every probe needs to be calibrated before being used, and the calibration has to be in the range of values to be measured for each variable.
Depending on the objectives of the study the depth resolution of the data has to be higher or lower, in waterbodies of more than 2-3 m it is recommended to obtain data every 20-30 cm (4 data points in 1 m). The stabilization time of the slowest probe gives the speed at which the multiparametric probe can acquire reliable data at different depths.

Light (PhotosyntheticallyActive Radiation, 400-700 nm) profiles also give much information about the physical and biological characteristics of the waterbody. These data will help to understand the balance between respiration and photosynthesis in the watercolumn, and also may help explaining the presence the submerged macrophytes. The most adequate way of measuring the light penetration in the watercolumn, is choosing a day with very low wind since the waves can distort the profile, and using simultaneously two flat sensors (cosine corrected), one at the surface level and the other underwater, facing upwards. For more accurate results 2 flat underwater sensors can be used, one facing downwards and the other facing upwards. 4 Pi sensors (spherical) give a more realistic set of data since they collect photons from all directions. In most cases high resolution is not needed and a measure every 30 cm or so should be enough to calculate the extinction coefficient. In extremely deep or turbid environments, it is necessary to find the depths at which 1% and 0.1% of the irradiance are reached, since these depths are considered important in terms of the photosynthetic capabilities.
The Secchi disk depth is also an interesting parameter that can be measured, although in many cases data cannot be extrapolated between different latitudes. The Secchi disk should be submerged in the water in the shade until the human eye cannot distinguish between the black and white triangles, this depth is called the Secchi depth.

7.3.2 Water chemistry

(Cynan Ellis-Evans, Antonio Quesada)

All the methods presented here have been in regular use for 20 years at Signy Island and, with the exception of Total Dissolved Nitrogen (TDN), Total Dissolved Phosphorus (TDP), Total Phosphorus (TP) and Total Iron, have also been used successfully in a field camp.

When working in a field situation we generally use pre-weighed components for making up reagent solutions. There are however small portable (battery-powered) top pan balances (e.g. Ohaus) that can also be used. We bring in distilled water in large containers or take water from oligotrophic lakes/streams, filter it and run it through deionising cartridges (but ensure the cartridges do not freeze). In the past we have run a mains powered spectrophotometer off a small Honda generator but we also ran a fan heater off the same generator to ensure a relatively high and constant load on the generator (and therefore more stable supply to the spec). Battery powered spectrophotometers or a combination of solar (or wind) powered battery packs and a power line inverter with a mains powered spectrophotometer are modern alternatives.

Sample Storage, Preservation and Preparation for Chemical Analysis

Storage and Preparation

If organic analyses are planned, borosilicate (Pyrex) bottles should be used to store samples, but otherwise use polythene or polypropylene bottles for sample collection. We favour Nalgene amber 1 or 2L polypropylene bottles as they are virtually unbreakable, light in weight, release few inorganic components (except trace metals) into the sample water, prevent photo-degradation of chlorophyll and algal activity, can be repeatedly frozen and are autoclavable. Polythene bottles are not autoclavable but remain flexible at sub-zero temperatures. Do not use soda-glass bottles unless unavoidable as these quickly release sodium, calcium and silicate into the sample water. For trace metal analyses use Teflon bottles. All new bottles should be acid-washed with 10% nitric or hydrochloric acid for 48h and this should be repeated after each sampling trip. Wash with deionised or deionised distilled water. Flush all bottles with sample water before taking a sample. If taking water samples in glass bottles have an insulated box for storage of the bottles during sample return, particularly in

winter. Always put bottles into an insulated box or rucksack to minimize wind chill effects as freezing disrupts pH, dissolved gases and silicate analyses. If possible do winter sampling inside a small tent to minimize solar radiation effects and to reduce wind chill effects on the sample hole and sample bottles (and the scientist!).

Return samples to the laboratory as quickly as possible (no more than a few hours) and filter (see below) the bulk of the water immediately to prevent interaction of particulate and soluble phases. Put aside a quantity of unfiltered water for Total P and Total N analyses. A further quantity of unfiltered water should be used for pH and alkalinity which must be analyzed before any other analyses. If possible, do the filtration in the field —but this usually requires a work tent or a field hut in close proximity to the study site. On return to the laboratory, always store filtered samples at <4°C and in the dark. It is highly desirable to undertake inorganic analyses the same day as sampling and certainly complete the analyses by the following day.

Preservation

If analyses cannot be undertaken on this time scale, preservation will be necessary. Freezing to -20°C is effective for certain ions, but is inappropriate for dissolved gases, pH and silicate (soluble reactive silicon) and there is some evidence of adverse effects on alkalinity and soluble reactive phosphorus. At Signy we also do not do ammonia-N analyses on frozen samples but other Antarctic groups do. Freezing seems to work well for organic carbon analyses —but for dissolved organic carbon pre-filter through ashed GF filters before freezing. Keep all samples cool and dark. If storage exceeds 2-3 days and freezing is not practicable, chemical preservation is possible, notably addition of chloroform (2.5ml per 500 ml of sample) or mild acidification, but in our experience these steps are undesirable as addition of chemical preservative precludes certain assays and can be inconsistent in its effect. The one exception we have observed is in the acidification of water samples for trace metal and cation (not ammonia-N) analyses (though it can disrupt ion speciation) where the method works well if samples are kept cool and dark.

Filter types

We have traditionally used acid-washed GF/C filters to filter water samples as they have a convenient pore size (~0.4µm) and fast throughput even at low vacuum, but the presence of significant cyanobacterial picoplankton in certain lakes require GF/F (~0.2µm) for accurate chlorophyll-a estimates to be made. Picoplanktonic particulates may also contribute an error to chemical analyses if the picoplankton are abundant. Filtration is a very slow business when using GF/F as minimum vacuum has to be applied to avoid cell lysis of freshwater protists. For general survey work I would still recommend GF/C for convenience. Some researchers use membrane filters but we find these clog up quickly and are too slow for filtering the numerous large volumes associated with our typical sampling trips. We use Mityvac hand vacuum pumps when in the field, if a powered vacuum pump is not available.

Suggested guidelines for treatment and storage of samples

Analyate	Storage conditions prior to manipulation	Initial Manipulation	Longer term Storage
pH and Alkalinity	<5°C, 24hrs maximum	Analysis	None
Ammonia-nitrogen	<5°C, 24hrs maximum	Filtration if turbid	Freezing may be OK
Nitrate-N	<5°C, 48hrs maximum	Filtration	Freezing
Nitrite-N	<5°C, 24hrs maximum	Filtration	Freezing
Total dissolved N	<5°C, 48hrs maximum	Filtration	Freezing
Total N	<5°C, 48hrs maximum	Analysis	Freezing
Soluble reactive P	<5°C, 24hrs maximum	Filtration	Freezing may be OK

Total dissolved P	<5°C, 48hrs maximum	Filtration	Freezing
Total P	<5°C, 48hrs maximum	Analysis	Freezing
Soluble reactive silicate	<5°C, 7 days	Filtration	None
Chloride	<5°C, 7 days	Filtration	Freezing
Sulphate	<5°C, 7 days	Filtration	Freezing
Sulphide	<5°C, 5 hours maximum	Analysis	None
Cations (Ca, Fe, Mg, Mn, K, Na, Li, Al, etc.	<5°C, 7 days	Filtration, acidification	<5°C
Dissolved organic C	<5°C, 48hrs maximum	Filtration	Acidification
Dissolved inorganic C	<5°C, 24hrs maximum	Analysis	None
Particulate organic C	<5°C, 48hrs maximum	Filtration	Freeze filter and store in dark

Tips

- Prevent water samples freezing in the field.
- If doing pH in the lab do this and alkalinity before filtration.
- Filter water samples through acid washed GF filters before inorganic analysis.
- Store samples cool and in the dark before analysis
- Complete inorganic analyses within 48h of sampling, ideally with 24h.
- If storing for long periods, freezing is the method of choice, but it is not perfect.

Sample Analyses

Laboratory measurement of pH

Many researchers undertake pH measurements in the field with handheld meters or as part of a profiling system but as Antarctic freshwater lakes are generally poorly buffered and environmental temperatures low, such measurements are often not that accurate (±0.2 pH units). Field meters often have relatively crude temperature compensation and typical pH buffer solutions to calibrate the probes are of very high conductivity compared to the samples. This results in considerable drift at low temperatures and if there is an interest in redox chemistry reactions such field measurements are generally unacceptable. With low conductivity calibration buffers and electronic detection of a stable signal accuracy improves to between ±0.1 and ±0.05. If very accurate pH values are required water samples are collected in glass bottles with ground-glass stoppers and quickly returned to the laboratory for pH measurements using a research grade instrument (±0.01 or better) with the sample in a narrow neck glass bottle to minimize atmospheric exchange and held at constant temperature to avoid use of automatic temperature compensation (ATC).

Total alkalinity

"BDH 4.5" Alkalinity

This is a very crude estimate of alkalinity but is a useful survey technique.

Reagents

a) 0.01M HCl - Ideally dilute a Convol solution. Can also dilute concentrated reagent grade acid followed by standardization against sodium carbonate standard.
b) BDH pH "4.5" Indicator solution (obtained from BDH Chemicals).
c) The sodium carbonate for standardization is prepared as 0.05M solution using reagent grade anhydrous sodium carbonate dried overnight at 110°C. Add 1.059 g to 1 litre of distilled or deionised water. The solution keeps best in polythene bottles.

Method

- Pipette 100 ml sample into a conical flask and add 10 drops of BDH 4.5 Indicator.
- Run in 0.01M HCl from a burette with continuous agitation of sample. Endpoint is reached when the blue colour turns through grey to pink.

Calculation
If v ml of acid is used in the titration then

$$\text{alkalinity (meq l}^{-1}) = 0.1 \times v$$

Note: to help with endpoint recognition a standard end-point colour can be prepared by adding 10 drops of indicator to a buffer solution of 50 ml 1M sodium acetate and 50 ml 1M acetic acid at pH 4.5. Keep in a stoppered conical flask and renew from time to time.

Gran Titration method *(after Mackereth et al., 1978)*
This has far more relevance for aquatic chemistry and for ^{14}C-primary production measurements than BDH 4.5 alkalinity, though the latter is a useful approximation for inter-comparisons. Three measurements are made whilst titrating between pH 4.4-3.7 and these are fitted to a Gran equation. Where the water pH is 6.0 or less there will be no free CO_2 and the three point titration measurement between pH 4.4—3.7 essentially yields total CO_2 rather than just alkalinity.

Reagents
a) 0.1M HCl. 8.61 ml of conc. HCl made up to 1 litre and standardised. The more convenient alternative is to use 0.1M Convol acid which only needs diluting in a stated volume of deionised/distilled water.

Method
Take a narrow-necked bottle and place a known volume (V_0) of water sample in it together with a magnetic stirring bar. The sample volume should occupy almost all the bottle volume. The narrow neck further minimizes gaseous exchange with the atmosphere. If possible, place the bottle in a recirculating constant temperature block or bath to ensure no temperature drift. Place a combined pH electrode at mid depth in the bottle and leave enough room to run in titrant from a high precision (ideally 0.005 ml resolution or better) piston burette (Metrohm) through a narrow bore (1mm) glass tube, the tip of which is located just above the stirring bar. There should be no air space in the glass tube. Start magnetic stirring (ensure it is stirring smoothly) and if necessary add known amounts of alkali to raise the pH to >8.5. Then run in known volumes (0.01-5.00 ml) of acid from the burette, noting the stabilised pH value after each addition. Modern pH meters will often indicate when the signal is stable, but otherwise allow to stand for 1 minute before further addition. Measure the pH values for three acid additions within the range pH 4.4 - 3.7, noting the volume of titrant added in each case. Determine the final volume of the sample and plot the results using the function F_2 derived from the equation:

$$F_2 = [\text{antilog } (a - \text{pH})] * (V_s + v)$$

where a = any convenient number (such as 5), V_s = initial sample volume and v = titrant volume (ml). $(V_s + v)$ corrects for dilution by the titrant. Plotting F_2 as the y axis against titrant volume v expresses the linear accumulation of free mineral acid after the end point. The alkalinity endpoint (v_2) can be determined as the point where the regression line for F_2 against v intersects the x axis. This value is then put into the equation

$$\text{Alkalinity } (\mu\text{eq l}^{-1}) = (0.100 * 10^6 / V_s) * v_2$$

Total carbon dioxide

Total carbon dioxide refers to the sum of all inorganic forms of carbonic dioxide. Similar to the above but requires two sets of three measurements during titration in the ranges pH 7.6-6.6 and 4.4-3.7. This yields two endpoints, v_1 and v_2 and v_2-v_1 is an estimate of total CO_2.

If the starting pH of the sample is below 7 as is often the case in Maritime Antarctic lakes then there are two options:

a) Carefully add 0.1M NaOH before acid titration to bring the sample pH up to ~7.6 and use this volume of NaOH for all other samples. If absolute original values of v_2 and v_1 are required, the V_s value must be corrected for the additions.

b) Use NaOH as the titrant. This is somewhat less satisfactory as pH response is slower than with acid, but can be used with care. Ensure the NaOH is made fresh each time to limit contamination by atmospheric carbon dioxide or store under a soda lime gas lock.

Reagents

a) 0.1M HCl. 8.61 ml of conc. HCl made up to 1 litre and standardised. The more convenient alternative is to use Convol 0.1M acid, which only needs diluting in a stated volume of deionised water.
b) 0.1M NaOH. Make up 0.4g of NaOH in 1 litre of water and standardise. More convenient is to use Convol 0.1M NaOH. Ideally make up fresh each time or store under a soda lime gas trap to prevent atmospheric contamination.

Method

Titrate as for alkalinity. The readings between pH 7.6-6.6 are used to calculate a Gran function

$$F_1 = [\text{antilog } (b - pH)]. (v_2+v)$$

which yields a linear plot against titrant volume v. Here b is any convenient number, such as 8. Intersection of the regression plot between F_1 and v gives v_1 (the free CO_2 acidity endpoint). Note: If 0.1M alkali is used for this titration (v_2+v) becomes (v_2-v).

The interval between v_1 and v_2 is the total CO_2 estimate. Total CO_2 in absolute concentration units is calculated from:

$$\text{Total } CO_2 (\mu\text{mol } l^{-1}) = (\text{molarity} \times 10^6 / V_S) * (v_2 - v_1).$$

Inorganic Nitrogen

Total Dissolved Nitrogen *(after Mackereth et al., 1978)*

Principle

Total nitrogen represents dissolved organic nitrogen, ammonia, nitrate and nitrite. There is a particulate N component in the water column but in terms of exchange with the soluble phase this is limited (unlike phosphorus). TDN (or TN if not filtered) is oxidised to nitrate and nitrite with alkaline persulphate digestion. After neutralisation, nitrate is reduced to nitrite, using spongy cadmium, and nitrite then determined spectrophotometrically.

Reagents

a) Oxidising solution. Dissolve 6 g of NaOH in 100 ml of distilled water and then dissolve 6 g of $K_2S_2O_8$(or 15 g of each chemical in 250 ml water). Make fresh daily.
b) 1M HCl - use Convol acid. This is stable for many weeks.
c) Buffer Solution. 75 g NH_4Cl in 400 ml of water. Adjust to pH 8.5 with ammonia solution and dilute to 500 ml. Stable for many weeks.

Method

- Filter water sample through GF/C filter.
- To 20 ml of sample in a boiling tube add 3 ml of oxidising solution.
- Cover with tin foil and autoclave at 15 psi for 30 min.
- Cool and then add 3 ml of 1M HCl to the digest.
- Transfer the digest to 100 ml flask, add 1.5 ml buffer solution and swirl to mix. Then follow the nitrate analysis given below.

Nitrate/Nitrite *(Mackereth et al 1978)*

Principle

Nitrate in solution is reduced to nitrite by spongy cadmium and nitrite determined spectrophotometrically.

Reagents

a) Spongy Cadmium - store in water. Rinse in 2% HCl for 15 min prior to use and wash three times with water. Repeat this procedure before returning the cadmium to its storage container after use. There is no need to throw spongy cadmium away after each run as it can be re-used many times. POISON. DO NOT DISPOSE OF SPONGY CADMIUM INDISCRIMINATELY.
b) 2.6% (w/v) NH_4Cl in aqueous solution
c) 2.1% (w/v) Borax solution in aqueous solution
d) 1% (w/v) Sulphanilamide in 10% vol/vol HCl
e) 0.1% (w/v aqueous solution) N.E.D. reagent. (N-1-naphthylethylenediamine dihydrochloride, aqueous solution)

Method

- Take 10 ml digest (or water sample if doing nitrate/nitrite not TDN) into 30 ml

polycarbonate Universal bottle (acid-washed).

- Add 3 ml NH_4Cl, 1 ml Borax solution and 0.5-0.6 g spongy cadmium (not critical). Place on a wrist shaker running at maximum speed for 20 min.
- Take 7 ml from the Universal (taking care to leave cadmium behind) and place in a second Universal.
- Add 1 ml sulphanilamide, mix and leave for 5 min.
- Add 1 ml of N.E.D., mix and leave for 10 - 120 min until colour development is complete. Colour is red and should emerge within a few minutes of standing.
- Dilute if necessary and read absorbance at 543 nm in a spectrophotometer. Run at least two replicates, 3 blanks and 3 standards with each set of samples.

For nitrite analyses, simply omit the spongy cadmium stage.

Ammonia *(after Mackereth et al., 1978)*

Principle
Ammonia reacts with phenol and hypochlorite in alkaline solution, when catalyzed by nitroprusside, to form indophenol blue.

Reagents

a) Phenol-Nitroprusside Reagent. Dissolve 15 g phenol and 0.015g sodium nitroprusside (added as 1 ml of 1.5% w/v aqueous solution, freshly prepared (1.5 g in 100 ml)) in 500 ml water. Stable for three months in a refrigerator.
b) Alkaline Hypochlorite Reagent. Dissolve 10 g NaOH in about 400 ml water and cool the solution. Add a volume of undiluted hypochlorite solution containing about 0.265 g chlorine (using Hopkins and Williams hypochlorite solution, 12% available chlorine, you add 2.4 ml to the above solution). Dilute to 500 ml, gently mix and store in a fridge. Stable for 3 months. Do not use hypochlorite with less than 8% free chlorine and do not use concentrated solution that has been stored for more than a year as the free chlorine content decreases over time.
c) Standard NH_4Cl Solution. Dissolve 3.281 g NH_4Cl in distilled water and make up to 1 litre. 1 ml = 1 mg NH_4-N. Working solution (10 $\mu g\ ml^{-1}$): Make 1ml of standard up to 100 ml with water.

Method

- Add 20 ml of sample with a pipette to ground glass stoppered test-tube. (Under field conditions where risk of atmospheric contamination low, I have successfully used a 30 ml Universal)
- Add 2 ml of phenol-nitroprusside, mix well, add 2 ml of alkaline hypochlorite and again mix well
- Place in a water bath at 25°C for 1 hour out of direct light, which is a source of significant interference.
- Read in a 4 cm cuvette at 635 nm, using distilled water and reagents blank.

Note: acid wash the tubes with 2M HCl between each run. When on a research station, run the distilled water used in reagent and standards preparation through a deioniser just before use to remove any contaminant ammonia from the distilled water.

We have found that we can replace sodium hypochlorite with sodium dichloroisocyanurate (DIC) which is more stable over time and less hazardous. Dissolve 0.2g of DIC in a solution of 1.6g analytical grade NaOH in 40ml of water. Add a solution of 20g analytical grade tri-sodium citrate in 40 ml water. Make up to 100ml with water.

Phosphorus

The phosphorus techniques are all based on the method of Eisenreich *et al*. (1975), *Environmental Letters* 9:1: 43-53.

Total and Total Dissolved Phosphorus

Reagents

a) Digestion Reagent. Dissolve 6 g $K_2S_2O_8$ in 80 ml deionised water containing 10 ml of 3.6N H_2SO4 and dilute to 100ml.
   Prepare fresh and discard after use. If heat is used to dissolve the persulphate allow the solution to cool before use.

b) Mixed Reagent. Mix 25 ml of

H_2SO_4-Antimonyl and 25 ml of Molybdate solution (see below) with 0.2 g of ascorbic acid in a 100 ml volumetric flask before diluting to 100 ml. Prepare for use and discard afterwards.

c) Standard Phosphorus Solutions. Dissolve 4.390 g of KH_2PO_4 in deionised water and make up to 1 Litre. 1 ml = 1 mg PO_4-P. Dilute standard x1000 to make working solution on day of use.

H_2SO_4-Antimonyl solution is prepared by mixing 53.3 ml of concentrated H_2SO_4 with 500 ml of deionised water and leaving to cool. 0.748g of potassium antimonyl tartrate ($K(SbO)C_4H_4O_6$) is dissolved in the diluted acid solution and the solution made up to 1 litre. Potassium antimonyl tartrate takes hours to dissolve. Leave until an opaque solution forms, then filter using Whatman No.1 filter paper.

The molybdate solution is made by dissolving 5.1495 g of sodium molybdate ($NaMoO_4$) in 250 ml of deionised water and diluting to 500 ml.

The digestion acid used in the digestion reagent is essentially 100 ml of conc. H_2SO_4 mixed with 500 ml of deionised water, cooled and diluted to 1 litre.

Method

- For TDP filter water sample through 0.45 µm filter before proceeding.
- Add 5 ml of digestion reagent to 25 ml of sample in a glass vial.
- Cover with aluminium foil and autoclave for 30 min at 15 psi.
- After cooling to room temperature, add 5 ml of the mixed reagent, mix and allow 15 min for colour development.
- Centrifuge for 10 min at 2500 rpm if a precipitate is visible.
- Measure absorbance at 882 nm in 4 cm cells against deionised water/reagent blanks.

Calculation of P content (assuming 4 cm cells)

Abs_{882} x (1/0.084) x (1000/25) = µg l^{-1}

Note - Total particulate P is obtained from TP - TDP.

Clean glassware is essential. Wash with Decon 90 (check for P), soak in nitric acid, wash in 2M HCl, followed by distilled, deionised water.

Dissolved Reactive Phosphorus

Reagents

As for TP/TDP except that there is no need for the digestion reagent.

Method

- Filter the sample through a GF/C filter and measure 25 ml into a glass vial.
- Add 5 ml of mixed reagent and allow 15 min for colour development.
- Read at 882 nm in a 4 cm cell against deionised water/reagent blanks.

Silicon

Soluble Reactive Silicate (after Mullins and Riley 1955)

Principle

Silicic acid and some derivatives react, in acid solution with molybdate to form yellow molybdosilicic acids which are then reduced to silico-molybdenum blues.

Reagents

a) Acid Ammonium Molybdate Solution. Shake 10 g of Analar ammonium molybdate with 350 ml of water, add 30 ml of Analar HCl and dilute to 500 ml. Store in a polythene bottle.

b) Reducing Agent Solution. Mix, in a plastic beaker, 100 ml of metol-sulphite solution with 60 ml of oxalic acid solution. Place beaker in a snow/water bath and carefully add 120 ml H_2SO_4 solution. Dilute to 300 ml with deionised water.

c) Standard Solution. Dissolve 0.6714 g Na_2SiF_6 (Analar) in 400 ml of deionised water, heating gently to avoid spattering until solution is complete. Cool and dilute to 1 litre before storing in thick walled polythene bottle. Solution contains 100 µg SiO_3-Si per ml. It is important that, before making this solution, the fluorosilicate should be dried at 80°C for 30 min, then transferred to a vacuum desiccator for 10 min at high vacuum before attempting to

weigh out the specified amount. The solution is stable for several weeks.

Note - Metol Sulphite solution is 6 g anhydrous sodium sulphite and 10 g metol dissolved in 500 ml of distilled water. The solution is filtered through Whatman No.1 filter paper and stored in a dark (aluminium foil is best) polythene bottle.

Note - Oxalic acid solution comprises 10 g Analar oxalic acid dihydrate dissolved in deionised water (heat to dissolve) and made up to 100 ml. Store in a polythene bottle.

Note - Sulphuric acid is a 25% solution (vol\vol) of sulphuric acid and distilled water. Store in a polythene bottle. Always add acid to water!!

Method

- Pipette 20 ml of sample (containing not more than 60 µg Si) into a 50 ml volumetric flask.
- Add 3ml of acid molybdate solution, cap and mix at several times over the next 10 minutes.
- Add 15 ml of reducing agent and make up to 50 ml with distilled water.
- Allow to stand for 3 hours and then read at 812 nm in a 4 cm cell. Include a deionised water/ reagents blank. Prepare a calibration curve for silicate - it should be linear.

We have done this analysis under field conditions, using 30 ml plastic Universals and halving the volumes quoted above without any discernible problems. Do not freeze water samples before undertaking silica analysis.

Chloride

We primarily use this parameter in maritime Antarctic lakes as a conservative measure of the concentration effect of ice exclusion when ice formation results in dissolved ions and gases being excluded from the freezing water, but it also clearly has relevance in ion balance studies.

Reagents

a) 0.2M Nitric Acid. Dilute 13 ml of conc (s.g. 1.42) Analar nitric acid to 1 litre using distilled water.
b) Mercuric Nitrate. Dissolve 3.4 g of $Hg(NO_3)_2.H_2O$ in 800 ml of distilled water to which 20 ml of 2M HNO_3 (2.6 ml conc. HNO_3 in 17.4 ml distilled water) has been added. Dilute to 1 litre. * HIGHLY POISONOUS*.
c) Diphenyl Carbazone-Bromophenol Blue Mixed Indicator solution. Dissolve 0.5 g of diphenyl carbazone and 0.05 g bromophenol blue in 100 ml of 95% ethanol. Solution is stable if kept in a brown bottle.
d) Sodium Chloride Standard. Dissolve 5.845 g of dry Analar sodium chloride in distilled water and make up to 1 litre. Contains 3.546 g of chloride per litre.

Method

- Add 10 drops of mixed indicator to 100 ml of sample containing not more than 50 mg chloride ion. At Signy, winter samples are diluted 1:1 with distilled, deionised water to save on use of mercuric nitrate rather than fall inside the 10 mg limit. [In highly reduced (anoxic) lakes add 3 drops of H_2O_2 (30%), as an oxidising agent, after adding the indicator.]
- Nitric acid (0.02N) is then administered dropwise until solution becomes yellow (pH 3.6) at which point a further 5 drops are added.

Titrate with mercuric nitrate solution to the point where the first tinge of purple blue appears and remains on shaking. The literature suggests an orange colour precedes the end-point but few workers have seen this. Using a magnetic stirrer helps but beware of highly toxic mercuric nitrate when changing stirring bars. Compare the endpoint against a distilled/reagents blank. A purple colour develops with time thereby invalidating comparing endpoints with a previous titration.

$$\text{Chloride (mg l}^{-1}) = \text{ml } Hg(NO_3)_2 \times 0.02 \times 1000 \times 35.46 \text{ ml of sample}$$

Sulphate

Principle
Ion chromatography is most frequently used for analysis of sulphate but a simple wet chemistry method is also available. This is based upon precipitation of sulphate as a barium salt and stablization of particle formation with gelatin. This is a much easier wet chemistry technique than methylene blue reduction.

Reagents
a) Barium chloride-gelatin reagent. Dissolve 0.6g of gelatin (Difco) in 200ml of hot 60-70°C water and allow the solution to stand at 4°C. After 24h bring the semi-gelatinous fluid to room temperature, add 2.0g of reagent grade barium chloride (dihydrate) and mix until the barium salt is dissolved. A second batch should be made to which barium chloride is not added and which functions as a colour correction control. Store the reagent in a refrigerator and always return reagent to room temperature (2hrs standing) before use. Make fresh every month.
b) 0.5M hydrochloric acid. Add 44.5ml of concentrated hydrochloric acid to about 300ml of water. Cool and make up to 1 litre with distilled water.
c) Potassium sulphate standard solution (1ml = 1mg SO_4^{2-}). Dissolve 0.9067g of potassium sulphate in about 400ml of water and make up to 500ml.

Method
- To 10ml of water sample, in a conical flask, add 1ml of 0.5M hydrochloric acid and 0.5ml of barium chloride-gelatin reagent.
- Mix and allow to stand for 30 min.
- Mix and read the spectrophotometer absorbance at 400nm.
- The influence of background colour can be corrected by subtracting the absorbance of another sample aliquot in the presence of barium chloride-free reagent. Linear calibration up to 25mg l^{-1} is possible. A calibration curve of 0-10mg l^{-1} gives a maximum absorbance of ~0.1 for a 1cm cell.

Sulphide

Principle
The sulphide present is precipitated as zinc sulphide by addition of zinc acetate. Sulphide and N,N, dimethyl-p-phenylenediamine are converted to methylene blue in the presence of ferric chloride and the colour change measured at 745 nm in a spectrophotometer.

Reagents
a) N, N, dimethyl-p-phenylenediamine sulphate. Dissolve 2g of salt in 100 ml of 50% (V/v) sulphuric acid. The reagent is stable for 1 month stored in the dark.
b) Ammonium ferric sulphate. Dissolve 18g of the salt in water and make up to 100 ml.
c) Standard iodine solution 0.025M. Dilute 50ml of 0.100M iodine (obtainable as Convol standard) in 150 ml of water.
d) Standard sodium thiosulphate solution, 0.025M. Dissolve 6.205g of sodium thiosulphate in 1 litre of water (or use a Convol standard). 1 ml of this solution is equal to 0.40mg sulphide ion.
e) Starch solution. Dissolve 1g of soluble starch in 100 ml water. Filter if necessary.
f) Hydrochloric acid 1M.
g) Standard sulphide solution. Add about 1g of sodium sulphide to ~800ml of oxygen-free water. Make up to 1 litre. This must be made fresh each time. Ensure bottle is full when sealed.
h) Oxygen free water. Vigorously bubble an inert gas (oxygen-free nitrogen) through a diffuser into distilled water for at least an hour.

Method
First standardize the sulphide solution. To 80 ml of distilled water in a 250ml conical flask mounted on a magnetic stirrer add 10 ml of HCl (reagent f), 10ml of iodine solution (reagent c) and mix. Using a 10ml burette add thiosulphate (reagent d), mixing carefully, until a pale yellow colour is obtained. Add a few drops of starch solution (reagent e) and continue titration until the blue colour just disappears. Record the titrant volume (v_1).

Prepare another flask containing 80ml of distilled water, 10ml of HCl, 10ml of iodine solution and, after mixing, add 10 ml of the sulphide solution (reagent g). Mix gently, cover the flask and leave for 2 mins. Titrate

the residual iodine with thiosulphate as before and record the volume of titrant (v_2).

Calculate the sulphide concentration from:

Sulphide standard ($mg.l^{-1}$) = (v_1 - v_2)/10 x 0.40

To prepare working standards, add 10ml of sulphide standard to a 500ml volumetric flask and dilute to the mark with oxygen-free water, mixing gently. Carefully fill a glass-stoppered bottle with this solution, avoiding trapped air bubbles and centrifuge for 15mins at 2500 rpm. Fill three 100ml volumetric flasks to the mark with oxygen-free water. Withdraw 5ml from one, 10ml from a second and 25ml from a third, replacing these volumes with corresponding volumes of diluted sulphide standard. Re-stopper and gently mix after each addition.

Add 1M zinc acetate [$240g.l^{-1}$] at a ratio of 2 ml of per litre of water sample to empty ground glass stoppered bottles and at the sample site fill these bottles with water sample before restoppering. On return to the laboratory, invert sample bottles to resuspend the precipitate, quickly transfer 100ml of water to a 100ml volumetric flask and stopper. To each flask, including standards, add 1 ml of reagent a, stopper immediately and mix. After 5mins add 1ml of reagent b and again mix. After 15 minutes measure the absorbance at 670nm in 1cm or 4cm cuvettes in a spectrophotometer. For sulphide concentrations above 250 ug l^{-1} dilute an aliquot with oxygen-free water , using the same procedure as in making dilute standards

Fe (II)

After Sorensen (1982).

Principle

The colorimetric reagent, ferrozine, binds both Fe (II) and Fe (III) but only Fe (II) gives a colour reaction. Fe (II) can therefore be measured in anoxic waters or sediments and reduction of Fe (III) to Fe (II) can be measured by the increasing presence of the latter. The colour reaction is very pH dependent so a buffer is necessary.

Reagents

a) Ferrozine reagent. Dissolve 0.02% ferrozine in 1 litre of 50mM HEPES buffer at pH 7.0.
b) 0.5M HCl.

Method

- Add 0.1-0.5 ml of anoxic water sample or culture sample or a known weight of sediment to 5 ml of 0.5M HCl and leave to stand for 1 hour at room temperature.
- Place 5ml of ferrozine reagent in a 25ml scintillation vial and transfer 0.05-0.2 ml of acid extract to the vial.
- Allow to stand for 15 minutes and then draw up the mixture in a syringe and push though a 0.2µm syringe filter into a cuvette, before reading absorbance at 562nm. A calibration curve can be constructed using ferrous ammonium sulphate. The buffering capacity of the ferrozine reagent will not handle more than 0.2ml of acid extract.

Total Iron

Iron can be measured relatively easily and selectively by atomic absorption spectrophotometry but this is also relatively insensitive for iron budgets. The alternative described here is a wet digestion followed by spectrophotometry with HCl. The evaporation technique used here gives the lowest contamination. The perchloric/nitric digestion is standard practice to remove organic matter but is hazardous.

Reagents

All reagents to be analytical grade

a) Water —double distilled and free of iron.
b) Hydrochloric acid, 5.93±0.07M. —Dilute 500ml of hydrochloric acid (sp. gr. 1.18) to 1L.
c) Hydrochloric acid, 1.1M±0.1M
d) Nitric acid, sp. gr. 1.42.
e) Perchloric acid, sp. gr. 1.70
f) Standard iron solution, 1000µg ml^{-1}. Dissolve 0.5±0.0001g of iron wire (99.998%) in 28ml of 8M nitric acid and make up to 500ml with distilled water.

Method

- Pipette 100ml of water into a 120ml

conical flask (acid washed with 5.93M HCl) and evaporate to dryness by heating overnight on a hotplate fitted with an aluminium alloy top and positioned under a 700W infrared heater (Radisil).

- Allow to cool and take the sample flasks to a fume cupboard for the wet digestion step.
- Add 0.5ml of perchloric acid and 1ml of nitric acid to each flask and wet any residue on the flask walls by very gently rotating the flask.
- Place on a hotplate and heat until fuming but do not allow to boil. Continue heating until the yellow-brown colour of organic matter is replaced by a pale yellow of iron.
- Fume off most of the remaining acid, allow to cool and add 3ml of 1.1M HCl, rotating the flask to wash down the walls and then fume off the acid on the hotplate.
- Allow to cool and dissolve the residue in 10ml of 5.93M HCl, again taking care to wash down the walls, and transfer the solution to a 50ml centrifuge tube and centrifuge at 300 rpm for 3min to remove suspended material. (e.g. silica).
- Measure absorbance of the tertrachloroferrate anion in a 1cm sipper cell at 360nm using distilled water as a reference solution. Calibration curves are linear from 0-3.5mg l^{-1}. An absorbance of 1.000 corresponds to 1.88mg l^{-1} of iron. Copper is the only significant interference and is negligible below 0.1mg l^{-1}.

Cations

Most elemental cations (Ca^{2+}, Mg^{2+}, Na^{+}, K^{+}) can be determined using highly sophisticated instruments, with very high precision. Typically atomic absorption (with grafite chamber or not) and Inductive Coupling Plasma are used.

8 Plankton description

8.1 VIRIOPLANKTON

(Eduardo Marco and Enrique Moreno)

In addition to the enumeration of viruses by electron microscopy, there are two main techniques for estimating the number of virus like particles (VLP) in aquatic environments by epifluorescence microscopy, using two different fluorochromes. For electron microscopy the samples are precipitated directly by ultracentrifugation on to the grid, and the typical staining procedures are followed.

SYBR-Green.

- Obtain a sample from the appropriate depth; 50 to 100 ml should be enough. Keep the sample in a dark bottle in darkness and cold. Fix the sample with glutaraldehyde or formaldehyde.
- The fluorescence procedure is based on the differential filtration through GFF, 0.22 and 0.02 µm pore size, and the staining of nucleic acids (both DNA and RNA) using the fluorochrome SYBR-Green (A and B). First we take the large particles out of the sample by filtering through a GFF filter. The filtrate only contains the small particles, i.e. some bacteria and most viruses.
- This filtrate is separated into two aliquots. One is passed through a 0.02 µm filter, which retains all the VLP and bacteria. The other is first passed through 0.22 µm, retaining most bacteria, and subsequently through 0.02 µm, retaining only the VLP.
- The filters are stained using SYBR-green A and B, and observed under epifluorescence microscopy under maximum magnification.

Yo-Pro 1.

- For quantitative virioplankton determination water samples are collected in dark bottles (50-100 ml).
- Samples are filtered with GF/F filters and subsequently Anodisc 25 membrane filters (0.2 µm pore-size) just before treatment or long term storage at 4°C. After this second filtration the bacteria population is practically removed. The sample can now be stored for nearly a year without changes in VLP concentration. But if samples are fixed with aldehydes, virus numbers decrease quickly (75% loss in four weeks, Xenopoulos & Bird, unpublished data).
- 100 µl of filtered water is diluted with 700 µl of prefiltered (pore-size 0.02 µm) deionized distilled water (DDW) and filtered through a 0.02 µm pore-size Al_2O_3 Anodisc 25 membrane filter (Whatman), with a premoistened GF/F as a backing filter, for a homogeneous distribution. In ultra-oligotrophic environments 1 ml of sample is diluted with 4 ml of DDW. In marine samples Xenopoulos & Bird rinsed three times, by filtering with 500 µl of prefiltered DDW.
- The filter is placed over 80 µl staining solution (Yo-Pro 1, 1mM (Molecular Probes), diluted to 50 µM in an aqueous solution of 2 mM NACN) in a (pyrex) Petri dish, with the sample side up, with a filter paper soaked with 3 ml of an aqueous NaCl solution (0.3% wt/vol).
- In the original method, as used by Hennes & Suttle (1995), samples were incubated in a covered Petri dish for 2 days, in the dark and at room temperature. Xenopoulos & Bird (1997) modified the protocol to reduce staining time, by using a microwave at intermediate power level (350 W) during 3.5-4 min. This modification of the method not only reduces the time necessary for staining, it also allows the use of aldehydes to fix the samples.
- After the heated Petri dish has cooled (10 min; if Xenopoulos´ protocol was used), the 0.02 µm membrane filter is rinsed three times with aliquots of 800 µl of prefiltered DDW on the filtering support. It is important not to let the filter dry out in this final step.
- Filters are placed with a drop of aquapolymount (Polysciences, Inc.) on a slide and covered with a cover slip and stored at -20°C until processed.

- For each sample, >200 viruses in 20 randomly selected fields were counted at a magnification of 1,000x in a epifluorescence microscope excited with blue light.

References

Hennes, K.P. and Suttle, C.A. 1995. Direct count of viruses in natural waters and laboratory cultures by epifluorescence microscopy. *Limnol. Oceanogr.*, 40(6), 1050-1055.

Xenopoulos, M.A. and Bird, D.F. 1997. Virus à la sauce Yo-Pro: Microwave-enhanced staining for counting viruses by epifluorescence microscopy. *Limnol. Oceanogr.*, 42(7), 1648-1650.

8.2 BACTERIOPLANKTON AND PROTOZOOPLANKTON

(Cynan Ellis-Evans)

Sampling and Fixation

The volumes of water needed for enumerating the various components of the microbial plankton vary significantly. The most oligotrophic lakes occur on the Antarctic continent and a reliable microscopical count will require 15-20ml for bacteria, 500-1000ml for phototrophic and heterotrophic flagellates and 1-2 litres for ciliates and heliozoan sarcodines. In oligotrophic maritime Antarctic or sub-Antarctic lakes 2-5ml for bacteria, 30-100 ml for flagellates and 500-1000ml for ciliates and sarcodines is usually sufficient.

A wide range of water sampling devices is available but many do not function well at sub-zero air temperatures so any new device should be first tested at realistic temperatures. The NIO sampling bottle is reliable and robust but takes only 1-2 L samples so would be too limited for some work. A perspex Kemmerer sampler of 5 litre capacity has been used extensively in continental Antarctic lakes as it has a simple, reliable trigger mechanism though it is not appropriate for close interval sampling due to its size (<1m long). It is also possible to use a manual diaphragm pump with rigid tubing or a battery-powered peristaltic pump. Pumps can collect large volumes but also create shear forces that can damage delicate protists so must be used carefully.

When obtaining water samples it is important to avoid exposure of samples to direct sunlight if at all possible as this stresses photoautotrophs. Ensure the samples do not freeze during transport to the laboratory. When samples will not be returned to the laboratory within a couple of hours it is recommended to have fixative placed in the sample bottles before going into the field. The same fixation regime is used whether fixed in the field or the lab. For bacteria and flagellates buffered gluteraldehyde added to 100 ml water samples, giving a final concentration of 2%, is adequate, whilst for ciliates a separate 1 litre sample fixed with Lugols Iodine (to give a final concentration of 1%) is employed. Keep samples cool and dark until analysed.

Fixatives for bacteria, phototrophic and heterotrophic flagellates

-Buffered glutaraldehyde

Buffer

$Na_2HPO_4.12H_2O$	7.16g
$NaH_2PO_4.2H_2O$	3.12g
Distilled water	100ml

The buffer components dissolve very slowly and should be mixed with a magnetic stirrer. Add 80ml of 25% glutaraldehyde to 20ml of the buffer and filter to remove particles. Store in a fridge until used. Add to samples in the ratio of 1:9 glutaraldehyde to sample volume.

Samples fixed with gluteraldehyde should be stored in the dark in the fridge. Bacteria can be stored indefinitely, while flagellate samples should be examined as soon as possible although some workers suggest that one may store samples for several months without appreciable loss of autofluorescence.

Water samples fixed with gluteraldehyde can be stored for some time cool and dark but it is important to ensure efficient ventilation particularly if the storage area is a room used by personnel for long periods, as the safe working limits for gluteraldehyde are less than 0.5 ppm. Where possible water samples should be filtered and preserved frozen on slides to remove any gluteraldehyde threat. If the water sample has to be maintained for a time it should be placed in a refrigerator to minimize the potential hazard.

Fixatives for ciliated protozoa (and large flagellates, e.g. dinoflagellates)

-Lugol' s Iodine

Iodine	10g
Potassium iodide	20g
Distilled water	200ml
Glacial acetic acid	20ml

Mix the iodine, potassium iodide and water together and then add the acetic acid. Dilute 1ml:100ml of sample. This methodology is cheap and, unlike gluteraldehyde, relatively non-toxic to users. However it does stain cells dark brown. They can be bleached to reveal internal structures by applying a 5% solution of sodium thiosulphate. Lugol' s iodine is an excellent fixative for oligotrichs and tintinnids (which are common in lower latitude lakes and in polar seas). This is considered by some the best routine fixative for measuring biovolumes, since it causes only about 5% shrinkage in cells. But others have reported up to 30% shrinkage in flagellates and oligotrichs so care should be taken when using Lugol' s iodine in drawing conclusions regarding biovolumes.

In the case of ciliates the Lugols iodine fixed samples are too bulky for convenient storage and so where possible are pre-settled in large measuring cylinders for 4-5 days, the bulk of the water discarded and ~50 ml of concentrate placed in amber plastic bottles and stored cool.

Alternative fixatives include:

Mercuric chloride
A 3-4% solution is exceptionally effective and it preserves good cell structure and does not cause excessive shrinkage. Its disadvantages are its expense where large quantities are used and its extremely high toxicity. There are serious problems with using this fixative in the Antarctic because of modern disposal regulations.

Formaldehyde
It is used as a 2% solution buffered for use in freshwater. This fixative causes shrinkage and distortion in many species.

Glutaraldehyde
This can also be used for ciliates as a 2% solution containing 2% tannic acid to reduce shrinkage especially in oligotrichs.

Cell Counting

-<u>Bacterioplankton</u>:
A suitable volume of water (see above) is pippeted onto a 0.2µm black Nuclepore (or Millipore Isopore) polycarbonate filter overlying an 8µm backing filter in a sterile filter apparatus. Add sterile filtered distilled water if necessary to ensure the entire filtering surface is covered evenly with sample before applying around 700µl of DAPI (1mg in 20ml distilled water) through a 0.2µm in-line syringe filter. Leave the sample to stand for 5 minutes and apply a gentle vacuum (<50mm Hg) to draw the sample through the filter. The filter is then removed and mounted on a microscope slide, a drop of immersion oil dispensed onto the filter, which is then covered by a cover-slip. In this form the samples can be stored dark and frozen for months. A photo-protectant such as Citifluor or Vectashield can also be added to the filters before placing the coverslip and these can significantly reduce photo-fading under fluorescence microscopy. Bacteria can be enumerated (and measured) in an epifluorescence microscopy preferably equipped with a 100W UV lamp. Magnification should be a minimum of x1250 and preferably 1500-1600 magnification, including an oil immersion lens. The eye-piece should be fitted with a Whipple grid and a drop of immersion oil placed on the coverslip. At least 500 cells (in a minimum of 15 views) should be counted.

-<u>Heterotrophic, Photoautotrophic and Mixotrophic Flagellates</u>:
The sample (volume will vary with season) is filtered through a 2µm pore size black

polycarbonate filter after staining with DAPI as described above. The filters are examined under epifluorescence microscopy switching between UV and blue filter sets to determine the number of phototrophic and heterotrophic flagellates (chlorophyll autofluoresces red under the blue filter whilst DAPI stained cells fluoresce under UV). At least 50 views and 200 flagellates per filter are counted.

-Ciliates:
Samples are concentrated by settling in 50ml settlement chambers for 4-5 days. The concentrated sample is then further concentrated by gentle centrifugation at around 800-1000 rpm. A final 1ml of concentrated sample is counted in a Sedgewick-Rafter counting chamber. At least 200 cells, or the entire chamber, are counted for each sample.

Calculation of Biovolumes

This is still far from precise as it depends to a very significant extent on conversion factors. In all instances at least 50 cells per filter are chosen randomly and measured with a line graticule or, if available, by an image analysis software package linked to a microscope camera. Mean cell volumes are calculated by using appropriate geometric formulae (eg. spheres, cylinders).

Bacteria —carbon biomass is estimated using the conversion factor of 350fg C μm^{-3} for bacteria (Bratbak G (1993) Microscope methods for measuring bacterial biovolume: epifluorescence microscopy, scanning electron microscopy and transmission electron microscopy. In: Kemp PF, Sherr BF, Sherr EB & Cole JJ, eds, Handbook of methods in aquatic microbial ecology. Lewis Publishing, pp. 309-317).

Flagellates —Carbon biomass conversion factors are 220fg C μm^{-3} for HNF (Borsheim & Bratbak 1987, Mar Ecol, Prog Ser. 36: 171-175) and 200fg C μm^{-3} for PNF (Wetzel, R.G. and G.E. Likens. 1991. *Limnological Analyses*, 2nd edition. Springer-Verlag New York, Inc).

Ciliates —The conversion factor used is 190fg C μm^{-3} (Putt, M. and D.K. Stoecker. 1989. An experimentally determined carbon:volume ratio for marine "oligotrichous" ciliates from estuarine and coastal waters. *Limnol. Oceanogr.* 34:1097-1104).

8.3 OBSERVING AND INVESTIGATING PROTOZOANS IN ANTARCTICA

(Wolfgang Petz)

Protozoans (protists) are widespread in almost all terrestrial and limnetic biotopes in Antarctica (e.g., Thompson 1972, Smith 1978, James et al. 1995, Ellis-Evans 1996, Petz 1997, 2003, Laybourn-Parry et al. 2001). Among these organisms, the flagellates are a phylogenetically rather diverse group, whose representatives are often separated into zooflagellates (colourless) and phytoflagellates (pigmented). In Antarctica, flagellates are widespread in fresh and saline water bodies, where they are often the dominant protozoan group, and some species live in soil (e.g., Smith et al. 1990, Laybourn-Parry et al. 1995, Perriss & Laybourn-Parry 1997, Butler et al. 2000, Roberts et al. 2000). Being heterotrophs (bacterivores, herbivores, omnivores, carnivores, detritivores, saprovores), mixotrophs or autotrophs, they occupy several trophic levels and thus play a major role in the truncated food webs of Antarctica (Laybourn-Parry 1997). Flagellates feed on bacteria, cyanobacteria, diatoms, other algae, other flagellates, ciliates, detritus, dissolved organic matter and some species are obligate anaerobes.

The ciliates are a diverse and monophyletic group, which play a major role in the biological transfer of energy and matter by linking the production to higher trophic levels (Porter et al. 1979). Most species are heterotrophs (bacterivores, herbivores, omnivores, carnivores, detritivores) consuming a wide variety of food (e.g., bacteria, cyanobacteria, coccolithophorids, diatoms, other algae, heterotrophic flagellates, ciliates, fungi, small metazoans, organic particles and dissolved organic matter) (Corliss 1979). Several taxa are functional autotrophs or mixotrophs (Lindholm 1985, Laval-Peuto & Rassoulzadegan 1988, Laybourn-Parry 2002). Ciliates thus occupy a number of trophic levels and contribute to nutrient regeneration by acting as producers, primary or secondary consumers or decomposers. In order to avoid considerable ecological and taxonomical simplifications, as often done by lumping ciliates into a few groups according to shape or size, specimens have to be identified to species level. The procedures presented here are standard methods in the investigation of edaphic or limnetic ecosystems and have been used in the Antarctic or Arctic before.

Like the flagellates, testate amoebae (testaceans) do not form a monophyletic group. However, they are often studied together because the species usually produce a resistant and permanent test made of organic material, secreted plates or foreign particles, which consists of a single chamber. Testate amoebae are abundant in soil and freshwater benthos, but some species occur in the plankton as well (Schönborn 2003). They are mainly bacterivores, herbivores, fungivores, predators of other protozoans or detritivores (Meisterfeld 2002). Due to their resistant shell, they were among the first protozoans to be investigated in Antarctica (e.g., Richters 1907, Penard 1913, Decloitre 1964, Heal 1965, Smith 1978, Todorov & Golemanski 1996, Petz 1997). Their ecological significance in aquatic ecosystems is not well researched yet.

An extensive list of literature on Antarctic protozoans is given in Annex 11.

8.3.1 Terrestrial protozoans

Sampling terrestrial ciliates

10 subsamples of moss, litter or soil usually from the uppermost layer (e.g., 0-3, 0-5 cm, divide samples according to soil layers; include debris but do not take green parts of plants) are collected with a corer or spatula, pooled and mixed in a sterile plastic bag. Transport at ambient temperature, e.g. in insulated box (do not freeze or warm, e.g. by solar radiation). The same bulk material can be used for investigating other protozoan groups.

Qualitative investigation of ciliates

For biodiversity or taxonomic studies, raw cultures are established according to the non-flooded Petri dish method in Petri dishes (10-20 cm in diameter) by adding CO_2-free mineral

water to fresh (preferably) or air-dried moss, soil and litter samples until saturation, i.e. 5-20 ml liquid drains off when Petri dish is tilted and material is gently pressed with a finger. Run off liquid is investigated every few days over a period of at least 4 weeks, e.g. on days 2, 6, 12, 20, 30 (Varga 1959, Foissner 1987). Do not close lid tightly to allow air circulation.

Always start the investigation of a sample drop without using a cover slip because ciliate shapes are often distorted by cover slip pressure. Later and for a more detailed investigation, add a small dab of vaseline to each corner of a cover slip and place it on the sample drop. If the cover slip is gently pushed down with a needle, the ciliates can be immobilized. A wet chamber (place moist filter paper in Petri dish) can be used to store living ciliates in a drop of water on a slide for a while, e.g. after isolation. For staining methods see limnetic ciliates below.

Counting terrestrial ciliates

Active ciliates are enumerated with a direct counting method in fresh samples as soon as possible (at least within 24 hours of sampling). Depending on ciliate density, 4 replicates of 0.1 g fresh soil (each consisting of 10 subsamples of 0.01 g from different parts of the bulk sample) are diluted with about 5 ml tap or commercial CO_2-free mineral water and examined on a slide without using a cover slip at 40x total magnification under a compound microscope (Lüftenegger et al. 1988a). Alternatively, soil extract medium can be used. Add the water immediately before counting. Specimens are identified to species if possible and abundances are given as individuals g^{-1} dry mass of soil or unit area^{-1} (cm^{-2}, m^{-2}). Fresh biomass is estimated volumetrically by approximating ciliate shapes to simple geometric bodies by using *in vivo* dimensions (measure at least 10 specimens per species) and assuming that 1 μm^3 = 1 pg protoplasm. To calculate carbon biomass, the factor for carbon content / fresh biomass = 0.0675 (Finlay 1982, Foissner et al. 1992). Densities of nematodes and rotifers (sometimes also tardigrades) can be assessed simultaneously.

In addition, soil moisture content and/or bulk density have to be determined according to standard procedures. Culture methods, e.g. most probable number (MPN) techniques, are inadequate for estimating active ciliate abundances (Berthold & Palzenberger 1995).

Sampling terrestrial testate amoebae

For quantitative and qualitative investigations, collect 10 subsamples of soil, litter or moss with a corer or spatula, pool in a sterile plastic bag and mix (as in ciliates, see above). Transport in insulated box and preserve and stain samples as soon as possible with phenolic aniline blue for enumeration of abundance.

Qualitative investigation of testate amoebae

For qualitative studies, air-dried soil is suspended in water in wide-mouthed containers and gently stirred. After about 15 min air-filled tests can be collected with a pipette from the water' s surface along the container' s periphery (Meisterfeld 1995). Initially, examine samples without a cover slip so that tests can be turned over with a mounted eyelash or they can be pipetted out. Methylcellulose (tylose; $C_5H_{10}O_5$)) can be used to increase the viscosity of the drop so that tests can be turned over and remain in a particular position. Species are usually identified by test shape and morphology.

Alternatively, air is gently pumped into the sample container using an aquarium pump, air-filled tests float up to the water' s surface where they accumulate on the underside of cover slips, which are suspended directly above the water level with a thin thread attached by plasticine (Schönborn 1966).

Abundance, biomass and diversity of testate amoebae are often studied in preserved material. Staining with phenolic aniline blue distinguishes between viable (active, cystic, precystic) and dead stages and thus can also be used to count specimens. Before investigating these samples, wash several times with distilled or deionised water until the blue colour disappears in the supernatant (use centrifuge).

Phenolic aniline blue stain

Fresh soil or sediment is fixed and stained in a plastic centrifuge tube (screw-cap); add at least an equal volume of fixative to the sample

(Lüftenegger et al. 1988a). Preserved samples can be stored at room temperature. If the blue colour fades in the supernatant, add phenolic aniline blue solution.

Reagents
Phenolic aniline blue: 150 ml 5% aqueous phenol (C_6H_6O), 10 ml 1% aqueous aniline blue, 40 ml glacial acetic acid. Filter and use undiluted; solution can be stored for years.

Albumen-glycerol: egg albumen and concentrated glycerol 1:1 (same as for ciliates in protargol impregnation, see below).

Counting terrestrial testate amoebae

A defined amount (e.g., 1-2 g) of fresh soil, litter or moss is fixed and stained with at least the same volume of phenolic aniline blue. Subsequently, the stained material is washed several times in distilled or deionised water using a centrifuge until the blue colour fades in the supernatant. Depending on testacean abundance, an equivalent of 0.1-0.5 g fresh soil is counted at x100 total magnification without using a cover slip (Lüftenegger et al. 1988a). Dilute sample with water as appropriate. In soils with a high humus content, add about 0.1 ml albumen-glycerol to 1 ml soil suspension in order to avoid excessive aggregation of soil particles. Specimens are identified to species; viable stages (or, preferably, active, precystic and cystic specimens) and empty tests are distinguished. Abundance is given as individuals g^{-1} dry mass of soil or unit $area^{-1}$ (cm^{-2}, m^{-2}). Fresh biomass is estimated volumetrically by approximating test shapes to simple geometric bodies (measure at least 10 specimens per species) and assuming that 1 μm^3 = 1 pg protoplasm. To calculate carbon biomass, the factor for the carbon content / fresh biomass = 0.0675 (Finlay 1982, Foissner 1993). Empty and viable tests are used for assessing species diversity. Abundances of nematodes and rotifers (sometimes also tardigrades) can be enumerated simultaneously in these samples.

An alternative counting method for testate amoebae has been proposed by Couteaux (1967, 1975; membrane-filter technique). However, these methods are unsuitable for naked amoebae, whose abundance cannot be reliably estimated at present.

Sampling terrestrial flagellates

Same as in terrestrial ciliates (see above).

Counting terrestrial flagellates

Same as in terrestrial ciliates (see above) except that 4 replicates of 0.01 g are examined at 200-300x total magnification using a cover slip. Phase contrast microscopy is advantageous when the sample is sufficiently thin. Abundance is given as individuals g^{-1} dry mass of soil or unit $area^{-1}$ (cm^{-2}, m^{-2}). Biomass is calculated as in ciliates (see above).

8.3.2 Limnetic protozoans

Sampling limnetic ciliates

The various benthic biotopes in ponds, lakes and streams should be repeatedly sampled during a season in order to follow the natural succession, which occurs in conjunction with environmental changes (Foissner et al. 1991). Thus, at the beginning of the austral summer an ice corer may be necessary to drill through an existing ice cover. For faunistic studies, it is essential to sample as many different biotopes and water bodies as possible. However, it is virtually impossible to determine the complete species inventory of a given water body, because only a portion of the ciliate species are active at a given time and some species are very rare.

Instead of collecting a mixed sample from the various biotopes, it is preferable to sample each biotope separately (e.g., cyanobacterial mats, green algal felts, epilithion, detritus, sandy sediment). For each sample, several subsamples are collected over a distance of about 50 m along the water body (in a stream preferably on both sides and across the bed) and put in a wide-mouthed plastic container (volume about 0.6-1 litre; opening about 9-10 cm in diameter). The container is filled up to about 3-4 cm with benthic material and river- or lake-water is added until the container is about 2/3 full (sufficient air has to remain). Always start downstream and work your way upstream.

Epilithion (aufwuchs) is scraped off of rocks or brushed off with a small hand-brush (alternatively, collect several smaller rocks). Sandy sediment (upper 1-3 cm) and detritus of lentic zones is collected with a spoon or the lid of the container. When the sediment is anoxic (black), sample this separately by thrusting a small glass or plastic tube directly into the sediment under water and close it. Avoid air in the tube.

Sample containers have to be kept cooled during transport. Do not let them freeze, or warm by solar irradiation. The samples have to be investigated as soon as possible for active species, ideally no later than 24 hours after collection.

An alternative sampling procedure is by exposing slides or other artificial substrates, e.g. polyurethane foam, on the bottom or suspended in the water column for at least about 2 weeks (Cathey et al. 1981). However, this method usually yields only a fraction of the actual community present (Foissner et al. 1991).

Planktonic ciliates are sampled for quantitative estimates with bottles from definite depths and unfiltered water is used. For qualitative investigations, a plankton net of mesh size 10(-20) □m can be used to collect also the smaller species.

Live observation of ciliates

Live observation of fresh material and raw cultures is usually essential for the investigation of ciliate species diversity and also for a correct species identification because important characters (e.g., body shape, colour, cortical granules, shape and size of extrusomes, movement) are lost during fixation and impregnation. Interference contrast microscopy (DIC; ideally) or bright-field illumination is used.

The sample container is opened in the laboratory, 2-3 cover slips are placed on the surface of the water so that they float, and then left to stand still for about 30 minutes. This usually yields many ciliates because the more aerophilic species move to the water' s surface and accumulate under the cover slips.

Subsequently, a cover slip is placed on a slide with the attaching drop facing down (use tweezers). Small plant pieces and 1 ml of sandy sediment or detritus (1 large drop on the slide) are examined separately. Preferably, use interference contrast microscopy (DIC) or bright-field illumination. The ciliates are identified to species if possible. Investigate each sample until no or only rarely additional species appear. For a more complete species inventory, water samples are stored in a refrigerator at about 4□C or at ambient temperature and repeatedly investigated over a period of about 4 weeks. Do not close lid completely to allow air circulation.

If the sample permits, a drop is investigated without a cover slip at first because ciliate shapes are often distorted by cover slip pressure. For a subsequent and more detailed investigation, add a small dab of vaseline to each corner of a cover slip and place it on the sample drop. If the cover slip is gently pressed down with a needle, the ciliates can be immobilized. A wet chamber (place moist filter paper in Petri dish) can be used to store living ciliates on a slide for a later, more detailed investigation.

Supravital staining with methyl green-pyronin

A small drop of 1% aqueous methyl green-pyronin (solution can be stored for years) is used to stain the nuclear apparatus and the mucocysts of most ciliates (Foissner 1991). A drop of the stain can be added at the edge of the cover slip and passed under it by using a filter paper at the other end. Investigate the sample immediately because specimens are killed eventually.

Protargol impregnation

Several silver impregnation methods, all of which require some experience, are available for staining ciliates. Protargol is the most common and most universal of these and generally well suited for limnetic (and soil) species. However, a few systematic groups, e.g. colpodids, or species do not impregnate well with this technique. Protargol

impregnation reveals the infraciliature, nuclear apparatus and fibres.

The modification of Wilbert (1975) requires few specimens, only a few chemicals and provides excellent results. Specimens are picked with a micropipette from the water sample, either under a compound or a dissecting microscope, fixed in concentrated Bouin or Stieve's fluid ($HgCl_2$, sublimate) for at least 30 min in a staining block (about 2 ml of concentrated fixative is sufficient), washed several times in distilled water, bleached with diluted aqueous sodium hypochlorite (NaClO, commercial concentration diluted at least 1:20-1:40, add drop by drop until specimens turn light grey to almost clear, bleach slowly; this is the critical step!), washed several times in distilled water, impregnated for 30-60 min at 60°C or over night at room temperature in 1-2% aqueous protargol solution (silver proteinate; sprinkle powder directly on sample liquid and let dissolve), developed in diluted 1% hydroquinone solution (add drop by drop and check impregnation under compound microscope, develop slowly), fixed for about 5 min with 2.5% aqueous sodium thiosulfate ($Na_2S_2O_3$; few drops), washed several times in distilled water, mounted with a droplet of albumen-glycerol on to a slide (mix albumen-glycerol and sample drop with a needle and spread in thin layer), air-dried, dehydrated with a graded series of alcohol (start with 70%, 5 min each), cleared twice in xylene and mounted in synthetic neutral mounting medium (e.g. Permount, Eukitt, Entellan).

The procedure is performed in a staining block under a dissecting microscope, solutions are changed with micropipettes. Optimum dilutions and treatment times depend on species and have to be determined empirically. Always add the sample to the fixative. An alternative to bleaching with NaClO is the commercial product Clorox (dilute 1:100-1:200); deionised water can be used instead of distilled water. A mounted eyelash can be used to orientate impregnated cells in the spread out albumen-glycerol drop. Samples may also be stored in the fixative and impregnated later in the home laboratory. Other protargol protocols are also available (e.g., Foissner 1991, Lee & Soldo 1992), a quantitative method was described by Montagnes & Lynn (1987, 1993).

Reagents
Bouin: 15 ml saturated picric acid ($C_6H_3N_3O_7$), 5 ml formaldehyde (CH_2O, commercial concentration), 1 ml glacial acetic acid ($C_2H_4O_2$). Mix components immediately before use (components can be stored).

Stieve's fluid (sublimate): 38 ml saturated $HgCl_2$ (dissolve 60 g $HgCl_2$ in 1 l boiling distilled water), 10 ml formaldehyde, 3 ml glacial acetic acid. Mix components immediately before use (components can be stored).

Hydroquinone solution: 5 g sodium sulfite (Na_2SO_3), 1 g hydroquinone ($C_6H_6O_2$), 94 ml distilled water. Discard when liquid is brownish.

Albumen-glycerol: Separate egg white from yolk of 3 eggs and shake by hand in Erlenmeyer flask until stiff foam is formed, allow to settle for 1 min, decant liquid into another Erlenmeyer flask and shake again, repeat this 2-4 times. Allow foam in all flasks to settle for 10 min, collect liquid (albumen), add the same volume of concentrated glycerol ($C_3H_8O_3$) and 1 small crystal of thymol ($C_{10}H_{14}O$), leave for 2 weeks and then decant and use clear supernatant (can be stored; old and distinctly viscous albumen-glycerol can be diluted with distilled water).

Silver carbonate impregnation

This quick procedure is only useful for some ciliates, in particular colpodids, hymenostomes, prorodontids and heterotrichs, and replaces the protargol method in these groups. It stains the infraciliature, nuclear apparatus and some cortical and cytoplasmic structures. Note that the cells are usually significantly distorted by this preparation.

Ciliates should be concentrated first on a slide by picking out with a micropipette. In the modification by Augustin et al. (1984), specimens are fixed for several seconds to 3 min with 4% formaldehyde (1-2 droplets; mix fixative and sample liquid only by circular motion of slide), impregnated with 1-3 droplets

of Fernández-Galiano' s fluid for 10-60 secs, placed on a pre-heated hot plate (60-80°C) until liquid is cognac-brown (keep slide in constant circular motion; check impregnation under compound microscope) and are then removed. The impregnation is stable for several hours and can be fixed with a droplet of 2.5% aqueous sodium thiosulfate ($Na_2S_2O_3$). As the procedure is performed on a slide, start with a small drop of sample water. Optimum treatment times, concentrations and amounts of chemicals depend on many variables and have to be found empirically. An alternative protocol is described by Wilbert (1983).

When performed in staining blocks under a dissecting microscope, permanent slides can be produced by fixing impregnated cells with 2.5% aqueous sodium thiosulfate ($Na_2S_2O_3$), washing several times in distilled water, mounting with a drop of albumen-glycerol on to a slide, air-drying, dehydrating with a graded series of alcohol, clearing twice in xylene and mounting in synthetic neutral mounting medium. Solutions are changed with micropipettes; deionised water can be used instead of distilled water.

Reagents
Fernández-Galiano' s fluid: 0.3 ml pyridine (C_5H_5N), 2-4 ml Rio-Hortega ammoniacal silver carbonate solution, 0.8 ml proteose-peptone solution, 16 ml distilled water. Mix components immediately before use in this sequence (components can be stored).

Rio-Hortega ammoniacal silver carbonate solution: 50 ml 10% aqueous silver nitrate ($AgNO_3$; store in brown bottle), 150 ml 5% aqueous sodium carbonate (Na_2CO_3; add little by little and stir), add 25% ammonia (NH_3) drop by drop until precipitate completely dissolves, add distilled water up to 750 ml. Solution can be stored in brown bottle for several years.

Proteose-peptone solution: 96 ml distilled water, 4 g bacteriological proteose-peptone (sprinkle on water, do not stir), 0.5 ml concentrated formaldehyde. Solution can be stored.

Albumen-glycerol: egg albumen and concentrated glycerol 1:1 (same as in protargol impregnation, see above).

Dry silver nitrate impregnation

This quick and easy method requires only few specimens and is used to reveal the silverline system and provides preliminary information on the infraciliature. It is particularly recommended for microthoracids, cyrtophorids, tetrahymenids, peniculids, peritrichs, euplotids and aspidiscids (Foissner 1991).

In the modification of Foissner (1991), a drop of the sample is placed on dry slides pre-coated with egg albumen, spread out with a needle, air-dried, impregnated for about 1 min with a few drops of 1% aqueous silver nitrate ($AgNO_3$; do not touch albumen with pipette), washed 3 sec with distilled water (let water run gently across tilted slide), air-dried, pre-developed by exposing for 5-60 sec to an electric light bulb (40-60 W, 3-10 cm distance), developed for 30-60 sec with a few drops of developer, rinsed gently for 5-10 sec in tap water, fixed with 2.5% aqueous sodium thiosulfate ($Na_2S_2O_3$) in a staining jar, rinsed gently for 5-10 sec in tap water, dehydrated twice in 100% ethanol (3 min), air-dried and mounted with neutral mounting medium.

Optimum treatment times, concentrations and amounts of chemicals depend on various variables and have to be found empirically. Thus, process several slides simultaneously if sufficient material is available. Tilt slides during drying. Albumen layer should turn brownish-black during development. When albumen is black, add some component A to developer and/or shorten pre-development; when it is brown, add component B and/or C and/or pre-develop longer. The impregnation fades when pre-developed with sunlight or under a UV-lamp. Saline samples should be diluted with distilled or deionised water. Do not use distilled water instead of tap water (alternatively, use lake or stream water).

Reagents
Egg albumen: Remove germinal disk and leave in open bottle for at least 20 hours; may be stored in closed bottle for 2-3 days. To coat slide, breathe on surface and apply with finger tip in very thin layer.

Developer: 20 ml component A, 1 ml component B, 1 ml component C. Mix

components immediately before use in this sequence; stable for 1-3 days, replace when dark brown or precipitate appears; components can be stored.

Component A: 1000 ml tap water (40°C), 10 g boric acid (H_3BO_3), 10 g borax ($Na_2B_4O^-$), 5 g hydroquinone, 100 g sodium sulfite (Na_2SO_3), 2.5 g methylamino-phenol-sulfate [metol, $(CH_3NHC_6H_4OH)_2.H_2SO_4$].

Component B: 100 ml distilled water, 0.4 g methylamino-phenol-sulfate, 5.2 g sodium sulfite, 1.2 g hydroquinone, 10.4 g sodium carbonate (Na_2CO_3), 10.4 g potassium carbonate (K_2CO_3), 0.4 g potassium bromide (KBr). Can be stored for about 6 months in brown bottle.

Component C: 10 g sodium hydroxide (NaOH), 100 ml distilled water. Can be stored for several years.

Fixation for scanning electron microscopy

Ciliates are preserved for about 30 min in Parducz' s fixative (ratio fixative:sample 1:1 - 2:1; add sample to preservative), washed at least 5 times in 0.05 M sodium-cacodylate buffer and stored in the buffer in the refrigerator. For further treatment see Foissner (1991).

Reagents
Parducz' s fixative: 4 ml 2% aqueous osmium tetroxide (OsO_4), 1 ml saturated aqueous sublimate ($HgCl_2$; see protargol impregnation above). Mix components immediately before use. Fixative is highly toxic!

0.05 M sodium-cacodylate buffer: 10.7 g dimethylarsinic acid sodium salt ($C_2H_6AsNaO_2$), 1000 ml distilled water; adjust to pH 7 with hydrochloric acid (HCl). Can be stored for several months in the refrigerator.

Counting limnetic ciliates

Preferably, active ciliates are enumerated *in vivo* in fresh samples, e.g. 4 replicates of 0.5 ml of water, detritus or sediment, on a slide without using a cover slip at 40x total magnification under a compound microscope (Lüftenegger et al. 1988a) or in a Kolkwitz-chamber. The sample volume examined depends on ciliate density and ranges usually between 1-5 ml. A smaller volume, i.e. about 1 ml, is sufficient when >5 ind. ml^{-1} occur. If necessary, the microscope stage and all equipment used to handle ciliates (e.g., slides) have to be cooled (ice bags). Abundance is given as individuals l^{-1}, g^{-1} dry mass or unit $area^{-1}$ (cm^{-2}, m^{-2}). Fresh biomass is estimated volumetrically by approximating ciliate shapes to simple geometric bodies by using *in vivo* dimensions (measure at least 10 specimens per species) and assuming that 1 μm^3 = 1 pg protoplasm. To calculate carbon biomass, the factor for carbon content:fresh biomass = 0.0675 (Finlay 1982, Foissner et al. 1992). An alternative live counting method has been described by Sime-Ngando et al. (1990). Pump-sampling is inefficient for quantitative purposes as many specimens are lost (James 1991).

Planktonic ciliates (whole water sample collected with bottle) can also be fixed in 3-4% sublimate ($HgCl_2$), 5-10% Bouin or 1-10% acid Lugol' s iodine (formaldehyde is inappropriate), left to settle for at least 24 hours and counted in an Utermöhl-chamber with an inverted microscope at 100-200x total magnification (Utermöhl 1958, Laybourn-Parry 1992). However, identification to species or even genus may be impossible and live counts provide higher abundances (e.g., Sime-Ngando et al. 1990). Always add the sample to the fixative. Prolonged storage, i.e. more than 6 months, distinctly reduces numbers of ciliates in preserved samples (Sime Ngando & Groliere 1991). Alternatively, the quantitative protargol stain (QPS) can be used (Montagnes & Lynn 1987, 1993).

Reagents
Acid Lugol' s iodine: 10 g crystalline iodine, 20 g potassium iodide (KI), 200 ml distilled water, 20 ml glacial acetic acid. Use in 1-10% final concentration.

Counting limnetic testate amoebae

A defined volume (e.g., 3-5 ml) of water, sediment or other benthic material is fixed and

stained with at least the same volume of phenolic aniline blue. Subsequently, the stained material is washed several times in distilled or deionised water using a centrifuge until the blue colour fades in the supernatant. Depending on testacean abundance, an equivalent of 0.1-0.5 g sediment or 0.1-0.5 ml of water is counted at x100 total magnification without using a cover slip (Lüftenegger et al. 1988a). Dilute sample with water as appropriate. Specimens are identified to species; viable stages (or, preferably, active, precystic and cystic specimens) and empty tests are distinguished. Abundance is given as individuals l^{-1}, g^{-1} dry mass or unit area^{-1} (cm^{-2}, m^{-2}). Fresh biomass is estimated volumetrically by approximating test shapes to simple geometric bodies (measure at least 10 specimens per species) and assuming that 1 μm^3 = 1 pg protoplasm. To calculate carbon biomass, the factor for carbon content:fresh biomass = 0.0675 (Finlay 1982, Foissner 1993). Empty and viable tests are used for assessing species diversity. Abundances of nematodes and rotifers (sometimes also tardigrades and gastrotrichs) can be enumerated simultaneously in the sample.

An alternative counting method has been proposed by Couteaux (1967, 1975; membrane-filter technique).

Reagents

Phenolic aniline blue: 150 ml 5% aqueous phenol (C_6H_6O), 10 ml 1% aqueous aniline blue, 40 ml glacial acetic acid. Filter and use undiluted; solution can be stored for years.

Albumen-glycerol: egg albumen and concentrated glycerol 1:1 (same as for ciliates in protargol impregnation, see above).

Permanent preparations of testate amoebae

Tests are mounted on to a slide pre-coated with a thin layer of albumen-glycerol, air-dried, dehydrated with a graded series of alcohol, cleared twice in xylene and embedded in synthetic neutral mounting medium. A highly refractile mounting medium as used for diatoms, e.g. Styrax, enhances the visibility of test plates (Meisterfeld 1995). Protargol impregnation (same protocol as in ciliates, see above) can be used to reveal the cell morphology and also increases visibility of morphological details of the test. Alternatively, specimens can be preserved with 3% formaldehyde or 70% alcohol (Schönborn 1966).

Scanning electron microscopy of testate amoebae

Tests are fixed with formaldehyde, repeatedly washed in distilled water, mounted on to a small glass slide pre-coated with a thin layer of albumen-glycerol, air-dried, mounted on to aluminium stubs and sputter-coated with gold (Lüftenegger et al. 1988b). Alternatively, tests can be directly air-dried on to aluminium stubs pre-coated with the adhesive Mixtion à Dorer Clarifeé (Lefranc & Bourgeoise, France). Let adhesive dry on stubs for at least 2 hours.

Counting limnetic flagellates

For estimating total numbers of benthic flagellates, active individuals are counted live as soon as possible following Madoni (1994): 3 replicates (9.6 µl) of a homogenously mixed sample are transferred to a Fuchs-Rosenthal chamber and specimens are enumerated along the diagonal of the counting grid, i.e. in 16 squares (the 256 squares correspond to 3.2 µl), at about 200x total magnification using bright-field illumination.

A quick method for estimating total abundance of planktonic flagellates is live counting according to Massana & Güde (1991). A whole water sample collected with a bottle is investigated as soon as possible by examining several drops of 1-10 µl on a slide without a cover slip at about 100x total magnification under dark-field illumination. Choose the volume of the drop so that it contains 1-5 individuals and counting time per drop is less than 30 seconds. Abundance is given as individuals l^{-1}, g^{-1} dry mass or unit area^{-1} (cm^{-2}, m^{-2}). Biomass is determined volumetrically as in ciliates (see above).

Alternatively, samples are fixed (e.g., with 4% glutaraldehyde or 2% formaldehyde) and

stained with DAPI (4' ,6-diamidino-2-phenylindole dihydrochloride) or other fluorochromes and examined using epifluorescence microscopy (Porter & Feig 1980, Sherr et al. 1993). This allows differentiation of phototrophic (including mixotrophic) and heterotrophic specimens.

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8.4 PHYTOPLANKTON

(Antonio Camacho)

Community structure:

- take a sample of 250 ml of the appropriate depth and introduce it in a dark bottle.
- Fix the sample with Lugol´s Iodine (see above) or buffered formaldehyde (final concentration 4%).
- Keep the sample at 4ºC in darkness.
- The analysis of the sample is made by the Uttermohl method, after precipitating the sample and counting in an inverted microscope.

Pigment composition:

- Filter enough water sample through a GF/F filter to obtain colour on it. In oligotrophic waters we may need to filter up to 1000 ml of water. Filtration should be performed under medium - gentle pressure to avoid the breakdown of cells.
- Keep the filter frozen and in darkness, taking note of the filtered volume.
- Lipofilic pigments (as chlorophylls and carotenoids) are extracted by cutting the filter in small pieces and introducing them in HPLC grade 90% methanol (5 to 10 ml depending upon the phytoplankton concentration). Vortex vigorously and keep overnight in darkness in cold. Centrifuge or filter through GFF. The supernatant can be used for measuring chlorophyll *a* with spectrophotometer (see above) or for doing pigment-taxonomy by HPLC.

8.5 ZOOPLANKTON

(Antonio Camacho)

For sampling the zooplankton large volumes of water should be filtered through 50-60 µm net. If a quantitative sampling is required the water volume has to be recorded. For qualitative sampling plankton nets can be used, towing the net slowly but at constant speed from the bottom. Certain plankton nets allow calculations of the volume filtered but this is not precise. When sampling the zooplankton one has to consider that the animals migrate up and down in the watercolumn, and that most of the animals may concentrate at specific depths even close to the water bottom.

Once the animals are collected. they are narcotised with some soda powder and then fixed with 4% (final concentration) formaldehyde.

9 Benthos description

9.1 BENTHIC DIATOM COMMUNITIES

(Manuel Toro)

9.1.1 Sampling

Streams
Streams may contain diatom communities living on different benthic habitats: epilithon (on stones), epipsammon (on sand), epipelon (in mud or silts) and epiphyton (on aquatic mosses). These habtiats are sampled separately.

- In each sampling station, three substretches of shoreline (5 m long) are selected along a stream stretch (20 – 30 m long) with environmental conditions (flow, depth, width, bottom substrate, slope, water velocity) representative of the selected stream.
- In each substretch, three stones are collected from a zone that is permanently below water level (> 20 cm depth in shallow streams), to avoid the influence of drying out events, and far from inflow streams.
- The surface diatom communities are detached from the stones by using a toothbrush and washing with a distilled water bottle on a funnel to collect the sample in a polythene bottle.
- The three subsamples from each substretch are put together in the same bottle, to get three composite samples from each stream station.
- Samples are preserved by addition of Lugol's Iodine (formaldehyde is also suitable).
- To take samples from epipsammon or epipelon diatoms, a plastic pipette or syringe is used to suck up the surface layer (1 mm) of a ≈5 cm^2 area in each substretch.
- Mosses are collected by hand and washed with distilled water to remove epiphytic diatoms.

Lakes
The epilithic diatoms are sampled in lakes as follows:

- Three stretches of shoreline (10-15 m long) are selected along lake perimeter.
- In each stretch, three stones are collected from the permanently submerged zone (>30 cm depth) to avoid the effect of drying out events, and far from inflow streams.
- Methodology for detaching and preservation of diatom communities is same that used in streams.

The diatoms living on the surface of the bottom (epipsammon +epipelon) are sampled along a transect to study the composition of surface sediment diatom communities in relation to depth. The transect is laid out on a lightest side of the lake, and is sampled from a boat, using a corer (modified Kajak type) to get three subsamples of surface sediment in each selected depth (Camerón, 1997). The depth intervals will depend of total depth of the lake (one sample at intervals of 1 m would be adequate).
If the benthic habitat is heterogeneous (silts, sands, gravels), an Ekman grab can be used to collect additional samples at each depth. Surface sediment (2-3 mm) from each core is subsampled, using an extruding device (Uwitec system) which allows the cutting of thin slides of sediment core. These sediment slides are preserved in the dark at 1-5 °C.

Sediment traps
Sediment traps are used to collect sinking diatoms and particles in the lake water column, to study the relationship between living diatom communities and the sediment accumulation rate and the record of those diatom communities in sediment.

Cylindrical traps, with 3 replicate cylinders, are suspended at two different depths (1 and 2.5 m above the lake bottom) close to the deepest point of each lake. High aspect ratio traps (height : diameter => 10) (Bloesch & Burns, 1980) are used in non turbulent lakes to avoid important resuspension processes. Samples collection will be done at monthly intervals during expedition and after winter period (10-12 months) in next expedition.

Subsamples from wet sediment collected in traps are examined at light microscope to count live/dead/broken cells to estimate resuspension rates and sources of material (Cameron, 1995). Sediment samples from traps is collected by filtering the water with sedimented material from cylinders through preweighed fibre glass filters (Whatman GF/F). Filters are frozen until laboratory analyses: dry weight, loss-on-ignition, pigments.

9.1.2 Preparation for diatom identification and counting

Diatom analysis preparation for identification at microscope will be done following Battarbee (1986). A subsample (0.05 g) of wet sediment is taken from the central part of core slices and diatom frustules are cleaned to remove organic matter with severe oxidation with H_2O_2 (30%) for 3-5 hours at 90 °C in precleaned (NaOH) test tubes. To stop bubble, some drops of HCl (50 %) are added. Then, cleaned sample is centrifuged at 1200 rpm for 4 min. Latter, the supernatant is discarded, distilled water is added, mixed, and centrifugation process must be repeated 3-4 times. Diatom-distilled water mixture is stirred and an aliquot (0.5 ml) is removed with a clean pipette for each subsample. An aliquot is placed on a round coverslip and allowed it to dry slowly on a hot plate (30 °C). Microscope slides are placed on a hot plate (130 °C), placing a drop of diatom mounting medium Naphrax on them. The coverslips are inverted and placed onto the drop of Naphrax and then, let Naphrax solvent be evaporated. Slides are observed under a microscope using phase-contrast illumination and magnification of up to x1000. Battarbee & Kneen (1982) will be followed for diatom counting with microsphere technique.

9.1.3 References

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Jones, V.J., Juggins, S. & Ellis-Evans, J.C. (1993). The relationship between water chemistry and surface sediment diatom assemblages in maritime Antarctic lakes. *Antarct. Sci.*, 5: 339-348.

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9.2 MICROBIAL MATS COMPOSITION

(Antonio Quesada)

The microbial mats can be described from chemical and biological point of view. Typically the microbial mats are described in terms of surface area rather than of volume. For this reason the microbial mat under study is sectioned in cores of known diameter. To obtain these cores several methods may be used. A section of a plastic syringe (10 ml) will give a core of close to 1 cm^2, but the diameter has to be measured carefully and the surface calculated. Metal (brass or stainless steel) corers can also be used. These are available in many sizes (from 2 to 30 mm in diameter), and a whole set may be purchased. The diameter chosen will depend upon the mat heterogeneity, but it is advisable to collect a number (5-10) of 1 to 2 cm^2 cores, covering most of the heterogeneity of the microbial mat. The heterogeneity in benthic systems is very much larger that that found in planktonic systems. This makes it necessary to collect a large number of replicates.
The microbial mat is a complete ecosystem in which most of the biological processes take place. In the microbial mat profile we may find a clearly laminated distribution with photosynthetic-rich layers and respiratory layers. In many occasions these different layers can be sectioned in situ with razor blades or other sharp instruments. If this is possible, the description of each layer separately is interesting.

9.2.1 Chemical composition

Chemically it may be interesting to determine the composition of the community, including the biological material, the sediment particles incorporated into the mat matrix and extra-cellular components, such as mucilaginous mater. Once the mat cores have been obtained, remove the big sediment particles with a pair of forceps, and place the cores on Whatman cellulose filter paper. Leave it to dry some minutes and place on another dry paper. Put the cores on filter paper in Whirlpack or Ziplock plastic bags and freeze them until analysis. Note the core diameter. In this protocol we will not consider the dissolved substances that are present within the mat, and which are typically very different from those in the surrounding water.

Once in laboratory, thaw the cores and dry them at between 45 and 55ºC to constant weight (usually overnight is enough). Grind manually in Eppendorf vials with a plastic pestle, taking care not to break up any stones and collect them, by passing the ground material by 500 microm Nytal filter.

Dry weight

The weight of the microbial mat per surface area is an important ecological value. After drying and grinding the core, keep it in desiccator overnight and weigh it in a desiccated balance (0.1 mg resolution is recommendable) at least 5 times. After being weighed pour the powder in a ceramic, pre-tared vial (as small as possible) and keep overnight at 550ºC. After letting the vials cool in a desiccator, weigh them in a desiccated balance. With these two measurements we will have the total weight of the core and the ash weight, and by substracting the first from the second we obtain the ash-free weight, which represents the biomass present in the core.

C and N

The C and N content can be measured using elemental analysis. Keep the ground core free of stones in a desiccator and introduce the maximum weight allowed by the elemental analyser in the metal capsule. The results obtained will be relative, in % of the weight. As we know the weight per surface area, we can subsequently estimate the amount of N and C in a given surface area.

P

For the analysis of the P content in the microbial mat we use the same procedure as for Total P in the planktonic fraction (see above), using a ground core.

9.2.2 Biological composition

9.2.2.1 Chlorophyll a

Chlorophyll a content is one of the most important variables for an estimate of the biomass of the photosynthetic biota in the microbial mat. If photosynthetic bacteria are the object of the research, it may also be interesting to determine bacteriochlorophyll. In situ extraction and determination is recommended. In case the photosynthetic pigments can not be measured soon after collection, the the material should be frozen immediately.

In situ: introduce the freshly collected core in 10 ml of 90% methanol, shake gently, not breaking the core, and leave in darkness at 4ºC (or lower) overnight. Take all the liquid with pipette and filter through GF/F in syringe filters. Measure absorbance at 665 and 750 nm. Add another 10 ml of methanol to the extracted core and extract again overnight. Repeat this procedure as many times as necessary, until no more pigment is extracted (depending on the community this may have to be done from 2 to 7 times on the same core).

In the laboratory: introduce the frozen core into 10 ml of 90% methanol, shake vigorously and sonicate in ice, until total disruption of the core, leave in darkness at 4ºC overnight. Centrifuge (10 minutes at 10.000 rpm). Decant the supernatant and measure absorbance at 665 and 750 nm. Extract the pellet again, and repeat this until no more pigment is obtained (typically after the second extraction only very minor pigment concentration is obtained).

Calculations:

$$\text{Chl a } (\mu g\ cm^{-2}) = (Abs_{665} - Abs_{750}) * 13.14 * \text{vol extract (ml)} * (1/\text{core surface } (cm^2))$$

9.2.2.2 Microstructure

It may also be interesting to make a taxonomic analysis of the mat components. Samples can be fixed in 4% formaldehyde immediately upon collection, if possible already at the lake shore, and kept in darkness at 4°C. If electron microscopy or confocal microscopy are going to be used on the samples, it is better to fix them with glutaraldehyde 2.5% in phosphate buffer pH 7 and keep the sample after fixing in phosphate buffer pH 7 in darkness and cold (4ºC) until inclusion and/or sectioning is carried out.

9.3 AQUATIC BRYOPHYTE VEGETATION

(Satoshi Imura)

In the Antarctic, aquatic bryophytes are known as an important component of lake bottom vegetation, but information about them is insufficient. Aquatic bryophyte vegetation is most vigorous between 3 to 5 m in depth, and is not found in shallower areas. Bryophytes are often found at the deep bottom of the lakes, up to 30 m in depth.

Prior to the sampling, the distribution of aquatic bryophytes in the lake has to be clarified by multiple sampling with a simple core sampler (about 30 mm in diameter), or, when the lake is shallow enough, using a waterglass. The structure of bryophyte vegetation varies between sites and with species composition. In the most prominent case, bryophytes form dense, pillar-like structures, over 30 cm in diameter and up to 60 cm high (Imura et al. 1999).

For the floristic study, a core sampler or small dredge sampler are used. But to collect the whole structure of the bryophyte vegetation, these instruments are not suitable. A grab sampler, (e.g. Ekman) of 20 or 30 cm square is necessary for this purpose. The most important defect of the Ekman sampler for field surveys is its weight. By using steel mesh in some parts of the dredge, you can decrease the weight of the sampler. Be very carefull, however, to prevent cross-contamination between lakes by fragments of the vegetation left in the mesh, spring or other parts of the sampler.

Samples collected from the lakes and streams should be packed in plastic bags or bottles, kept in a cold and dark place, and frozen at –20ºC as soon as possible. It is important not to wash the bryophyte shoots before packing. Aquatic bryophytes provide a unique habitat to epiphytic algae (cyanobacteria, diatoms, or green algae) and numerous invertebrates.

Reference

Imura, S., T. Bando, S. Saito, K. Seto & H. Kanda (1999). Benthic moss pillars in Antarctic lakes. *Polar Biology* 22: 137-140.

10 Sampling and identification of protists

(Paolo Cavacini)

10.1 INTRODUCTION

Identification of algae is one of the main problems for scientists involved in biological and ecological studies on Antarctic freshwater ecosystems. Although it is often difficult to find macroscopic growths, microalgae are widespread in almost all terrestrial and freshwater environments of Antarctica (Seaburg et al., 1979, Broady, 1996, Jones, 1996). Over 813 species have presently been recorded for Continental Antarctica (Cavacini & Fumanti, 2001).

Algal species are present in fresh and brackish water of ponds and lakes of the coastal part of Antarctica and the sub-Antarctic Islands, and also in meltwater streams. In the latter environment the availability of liquid water - necessary for life - can dramatically change in a short period of time during the austral summer, resulting in ther death of the algal community, or inducing the algae to produce resting stages. Determination of the microalgal species in the limnetic environments is the first step for ecological and ecophysiological studies in such habitats.

In this account a number of methods for sampling and determination of the algal content of Antarctic limnetic habitats is given, taken from the available literature. This is not "the protocol" but a suggestion of a series of methodologies that can be followed or improved.

10.2 SAMPLING OF LIMNETIC HABITATS IN ANTARCTICA

Microalgal sampling of ponds, lakes and streams should be repeated several times within a single season in order to follow the natural variation of the algal population with seasonal meteorological changes (Laybourn-Parry et al., 1991, Oppenheim, 1994). At the beginning of the austral summer, lakes and most of the ponds in Antarctica are usually ice-covered. An ice corer is necessary to drill the ice and reach the water and the bottom of the lakes (Wharton et al. 1983).

Planktonic algae

Planktonic algae are present especially in the large, almost all year ice-covered Antarctic lakes (Laybourn-Parry et al., 1991). Non quantitative plankton samples can be obtained through the ice drill by vertical hauls with a 25 µm mesh phytoplankton net (Seaburg et al. 1979). During the favourable season the same net can be used from a small boat if ice cover is absent. The samples can be fixed with Lugol's solution (1/10 v:v), formalin (1/20 v:v) or can be stored at + 4 °C or –20 °C for immediate or later microscopic examination.

Quantitative samples for phytoplankton cell counts can be obtained by taking a known volume of 1-2 l of water at the surface level with a bottle (Seaburg et al., 1979). In deeper lakes sampling at different depth can also be done with a bottle (APHA, 1971, Venrick, 1978, Laybourn-Parry et al., 1991). The samples must be fixed after the collection.

Benthic algae

Benthic algal mats as defined and classified by Wharton et al. (1983) and Parker & Wharton (1985) are widespread in freshwater and saline ponds and lakes of Antarctica. They usually grow along the shores and on the bottom of the basins. Samples of shore mats can be obtained using sterilized instruments and sterile plastic bags or tubes (Holm-Hansen, 1964; Ling & Seppelt, 1998) or using small corer of 2,5 cm^2 (Howard Williams et al., 1990) or 7 cm^2 (Fumanti et al., 1997) depending on the mats thickness. Mats adhering to the bottom or pieces floating free in the water can be sampled by scuba or snorkel divers that can

sample the bottom (Oppenheim, 1994) or catch the floating mats in sterile plastic tubes (Wharton et al., 1983). A corer for algal mat sampling was used also by Ellis-Evans (1982). See also the chapter above on microbial mat sampling.

Counting planktonic and benthic algae

Planktonic algae can be counted using Utermöhl plate chambers after 24 hours of settlement of different amounts of water sample (10, 25, 50, 100 ml, depending on the plankton concentration in the sample) in top sedimentation cylinders (Hasle, 1978a). An invertoscope equipped with 32X or 40X objectives can be used to count the settled algae in a number of transects of the plate chamber (Hasle, 1978) or reach the count of 100 specimens for the most frequent species. Benthic algae can be counted using fluorescence methods (Walsby & Avery, 1996; Congestri et al., 2000).
Bacillariophyta samples should be cleaned with HCl and sulphuric-nitric acids mix (1:1 v/v) in order to obtain empty diatom frustules (Fumanti et al., 1997). Other stronger cleaning methods are available from the literature (i.e. Hendey, 1964) and the choice depends on the amount of organic material (usually very high in Antarctic algal mats). Cleaned material is used to make permanent slides in different mountant media. The most commonly used are: Hyrax (R.I. 1,71) (Kellogg et al. 1980, Oppenheim, 1994, Fumanti et al., 1997, Spaulding et al., 1999) or Naphrax (R.I. 1,69) (Schmidt et al., 1990, Hodgson et al., 2001). A list of mounting media is available in Reid (1978). 400 or 500 valves can be counted in each permanent slide with a light microscope at 1000 magnification (Oppenheimer, 1990, Roberts & McMinn, 1999), in order to obtain the percentage of each species in the sample. Another counting method for Bacillariophyta is used in Fumanti et al. (1995). A known surface (or weight) of algal mat is cleaned with acids to obtain empty diatom frustules. A known solution of ethanol-distilled water is prepared with empty frustules, and 1-2 ml of this is settled in an Utermöhl plate chamber and diatoms are counted as reported above in order to obtain frustules/cm^2 or /g of the mat.

10.3 IDENTIFICATION OF ALGAE

For a taxonomic analysis of planktonic and benthic algae a number of techniques are available. It requires taxonomic specialists if at all possible. Best results can be obtained combining direct observation and the use of unialgal cultures on different culture media.

10.3.1 Direct observation

Fresh material should be examined as soon as possible, using a good microscope equipped with 100x oil immersion objective and micro photographic apparatus. Information on morphology and pigmentation, frequency, number, and life cycle stages of the species can be observed in this way. This provides very useful information for identification. Subsequently the samples can be fixed, to allow taxonomists to confirm the determinations, or for comparison with other samples.

The main algal divisions recorded in Antarctic limnetic systems are Cyanobacteria (Cyanoprokaryota), Bacillariophyta (Diatoms) and Chlorophyta for benthic samples, and also Cryptophyta for planktonic samples. An account of the main taxonomic book useful for algal determinations is given in the following section.

10.3.2 Unialgal cultures

For species that can not be easily defined with morphological features only, it is necessary to obtain unialgal cultures, in order to follow the life cycle of the species. In particular many species of Chlorophyta show similar morphological features during their life cycle and the continued observation of unialgal cultures is necessary to assign specimens to different genera or species. Inocules from algal mats can be obtained from subsamples placed directly or after potter grinding in liquid and agarized culture media. Every 10 days cultures should be examined and microalgae isolated to obtain unialgal cultures (Cavacini, 2001). Many studies are based on unialgal cultures (Mattox & Bold, 1962; Cox & Bold, 1966,

Baker & Bold, 1970), also in Antarctic fresh and brackish water (Holm-Hansen, 1964, Seaburg et al., 1981,Tang et al., 1997). A very useful account of culture techniques is given in Stein (1983).

Culture media are numerous and all are suitable for developing Cyanobacteria and algae. The most used ones at present are BBM (Bold's Basal Medium, Chantanachat e Bold, 1962) and BG11 (Stanier e Cohen-Bazire, 1977) The recipes of these culture media are available at several internet-sites, e.g. http://www.ife.ac.uk/ccap/MediaRecipes.html

Bold's Basal Medium (BBM)

This medium is suitable for the cultivation of Chlorophyta and Cyanobacteria, also those isolated from limmetic systems (Akiyama, 1979, Broady, 1982, Nozaki & Ohtani, 1992, Fumanti et al., 1995, 1997, Ling & Seppelt, 1998).

BG-11

This medium was developed specifically for the cultivation of Cyanobacteria, but it will also successfully support the growth of a range of Chlorophyta and Xanthophyta. In Antarctica good results were obtained by Holm-Hansen, 1964, Fumanti et al., 1997, Tang et al., 1997.

Algal growth in culture can be induced or reduced by several physico-chemical parameters such as light and temperature. Several studies were carried out on temperature tolerance and optimum of microalgal Antarctic species. Almost all these studies clearly show that growth rates are highest at temperatures between 8-20°C for Cyanobacteria (Tang et al., 1997), Diatoms (Watanuki, 1979) and other algae including Chlorophyta (Seaburg et al., 1981). Some attempts were made to define the optimum light regime. Almost all Antarctic species can grow better with light-dark cycles of different ratio:

12:12 light/dark (Broady, 1992, Akiyama, 1979);
14: 10 l/d (Nozaki & Ohtani, 1992);
16: 8 l/d (Darling & Friedmann, 1987; Broady et al., 1997).

Continuous light does not seem to be suitable for best algal growth, probably because of photoinhibition (Broady, 1992).

10.3.3 Useful literature for algal determination

The "classic" approach of algal determination is based on morphological and life stage diversity of the observed specimens. In the last decade, an approach based on biomolecular markers (e.g. rRNA) started to change the classification systems (Brambilla et al., 2001), but it still needs to be expanded to all algal species. The "classic" approach is based on dichotomous keys that split specimens following progressively finer morphological and reproduction differences. The following list offers the general and specific taxonomic books for the main Algal divisions present in the Antarctic limnetic systems. No single comprehensive book on algal classification is available. Usually only one single division (or "group" as in the case Chlorophyta) is treated in each publication. More useful literature references are listed in Annex 10.

General Texts

These texts aid researchers that face for the first time the problem of the identification of algae. They are useful for classification at the family or genera level but they are not suitable for more accurate determinations.

Bourrelly, P. (1970) : Les algues d' eau douce. Initiation à la systématique. Tome III: les algues bleues et rouges. Les Eugléniens, Peridiniens et Cryptomonadines. Éditions Boubée, Paris, 512 pp.

Bourrelly, P. (1972) : Les algues d' eau douce. Initiation à la systématique. Tome I: les algues vertes. Éditions Boubée, Paris, 572 pp.

Bourrelly, P. (1981): Les algues d' eau douce. Initiation à la systématique. Tome II: les algues jaunes et brunes. Chrysophycées, Phéophycées, Xanthophycées et Diatomées, Société nouvelle des éditions Boubée, Paris, 517 pp.

Prescott, G.W. (1978): How to know the freshwater algae, 3rd edition, Wm. C. Brown, Dubuque. 293 pp.

Cyanobacteria

The classification of this group was recently partially revised by Anagnostidis & Komárek (see references below), who added to the morphological features also elements of ecology and molecular biology for the determinations. The result is that several new genera were erected in the last decades and some species were transferred from one genus to another. A completely opposite direction was followed by Drouet (1981), who tried to reduce the number of species, considering most of them as ecophenes of few different species. The obtained classification system was used by some authors, also in Antarctic studies (Zaneveld, 1988), but in the light of modern studies is now clear that such a simplification is not correct and this system should not be followed (Broady 1992).

Anagnostidis, K., Komárek J. (1988): Modern approach to the classification system of Cyanophytes 3 - Oscillatoriales. Arch. Hydrobiol. / Suppl. 80, Algological Studies, 50-53: 327-472.

Desikachary, T.V. (1959): Cyanophyta. I.C.A.R. Monographs on Algae, New Delhi. 686 pp.

Geitler, L. (1932): Cyanophyceae. In: L. Rabenhorst (Ed.), Kryptogamen-Flora von Deutschland, Österreich und der Schweiz. Bd. XIV. Acad. Verlag., Leipzig. 1196 pp.

Komárek, J., Anagnostidis, K. (1986): Modern approach to the classification of Cyanophytes 2 - Chroococcales. Arch. Hydrobiol. / Suppl. 73, 2 (Algological Studies 43):157-226.

Komárek, J., Anagnostidis, K. (1989): Modern approach to the classification of cyanophytes 4 - Nostocales. Arch. Hydrobiol. / Suppl. 82, 3 (Algological Studies 56): 247-345.

Komárek, J., Anagnostidis, K. (1999): Cyanoprokaryota. 1 Teil: Chroococcales. H. Ettl, J. Gerloff, H. Heynig & D. Mollenhauer (Eds), Süßwasserflora von Mitteleuropa. Bd. 19/1. G. Fischer-Verlag, Jena, Stuttgart, Lübeck, Ulm, 548 p.

Starmach, K. (1966): Cyanophyta - sinice, Glaucophyta - glaukofity - Flora slodkowodna Polski, 2. 808 pp.

Chlorophyta

Unfortunately a comprehensive text on the taxonomy of this division is not yet available, but several authors studied different ”groups“. Among them the following ones are the most complete and useful.

Ettl H. (1983): Chlorophyta I. Phytomonadina. In H. Ettl, J. Gerloff, H. Heynig, D. Mollenhauer (Eds), Süßwasserflora von Mitteleuropa. Bd. 9. G. Fischer Verlag, Stuttgart, New York. 807 pp.

Ettl H., Gärtner G. (1988): Chlorophyta II. Tetrasporales,Chlorococcales, Gloeodendrales. In H. Ettl, J. Gerloff, H. Heynig, D. Mollenhauer (Eds), Süßwasserflora von Mitteleuropa. Bd. 10. G. Fischer Verlag, Stuttgart, New York. 436 pp.

Ettl H., Gärtner G. (1995): Syllabus der Boden-, Luft- und Flechtenalgen. G. Fischer Verlag, Stuttgart, Jena, New York. 721 pp.

Komárek J., Fott B. (1983): Chlorophyceae: Chlorococcales. In: A. Thienemann (Ed.), Das Phytoplankton des Süßwassers. 7 Teil, 1 Hälfte. Schweizerbart, Stuttgart. 1044 pp.

Fott B. (1972): Chlorophyceae (Grünalgen) Ordnung: Tetrasporales. Das Phytoplankton des Süsswassers 16, 6. 116 pp.

Printz H. (1964): Die Chaetophoralen der Binnengewässer. Hydrobiologia, 24 (1-3): 1-376.

Bacillariophyta

Diatoms are distinguishable by their unique morphological features, especially for the frustule ornamentations. The use of instruments like the Scanning Electron Microscope (SEM), which can reveal finer structures and differences, has restricted the species concept in diatom taxonomy. This results in changes in the rank and position of some species and genera and sometimes even families in the diatom classification systems. The older and complete approaches, like those of Hustedt or Krammer & Lange-Bertalot (see references below) are now complemented with new observations started in the nineties by Round et al. (1990) and by H. Lange-Bertalot’s

Iconographia Diatomologica series (see references below).

Hustedt, F. (1927-66): In: L. Rabenhorst (Ed.), Kryptogamen-Flora von Deutschland, Osterreich under Schweiz, Vol. 7, Akademische Verlagsgesellschaft, Leipzig.

Krammer, K., Lange-Bertalot H. (1986): *Bacillariophyceae*. 1 Teil: *Naviculaceae*. In: H. Ettl, J. Gerloff, H. Heynig & D. Mollenhauer (Eds.), Süßwasserflora von Mitteleuropa Bd. 2/1. G. Fischer-Verlag, Jena. 876 pp.

Krammer, K., Lange-Bertalot H. (1988): *Bacillariophyceae*. 2 Teil: *Bacillariaceae*, *Epithemiaceae*, *Surirellaceae*. In: H. Ettl, J. Gerloff, H. Heynig & D. Mollenhauer (Eds.), Süßwasserflora von Mitteleuropa Bd. 2/2. G. Fischer-Verlag Jena. 596 pp.

Krammer, K., Lange-Bertalot H. (1991a): *Bacillariophyceae*. 3 Teil: *Centrales*, *Fragilariaceae*, *Eunotiaceae*. In: H. Ettl, J. Gerloff, H. Heynig & D. Mollenhauer (Eds.), Süßwasserflora von Mitteleuropa Bd. 2/3. G. Fischer-Verlag, Stuttgart, Jena. 576 pp.

Krammer, K., Lange-Bertalot H. (1991b): *Bacillariophyceae*. 4 Teil: *Achnanthaceae*, Kritische Ergänzungen zu *Navicula* (*Lineolatae*) und *Gomphonema*. In: H. Ettl, J. Gerloff, H. Heynig & D. Mollenhauer (Eds.), Süßwasserflora von Mitteleuropa Bd. 2/4. G. Fischer-Verlag Jena. 437 pp.

Lange-Bertalot H. (1995-2000): Iconographia Diatomologica. Vol 1-9. Koeltz, Koenigstein.

Round, F.E., Crawford, R.M. and Mann, D.G. (1990): The Diatoms. Biology and morphology of the genera. Cambridge University Press, Cambridge. 747 pp.

Other groups

Species belonging to Xanthophyta and Cryptophyta have been found in Antarctic freshwater and brackish waters. Xanthophyta are present in meltwater streams, where water availability can dramatically and rapidly decrease leading to dry conditions that favour the development of species of this division. Cryptophyta occur in lake phytoplankton, especially with *Chroomonas* (*Rhodomonas*) *lacustris* Pascher & Ruttner (Seaburg et al., 1979).

Ettl H. (1978): *Xanthophyceae*. In: H. Ettl, J. Gerloff & H. Heynig (Eds.), Süßwasserflora von Mitteleuropa. Bd. 3, I Teil. G. Fischer Verlag, Stuttgart, New York. 530 pp.

Huber-Pestalozzi G. (1950): Cryptophyceen, Chloromonadinen, Peridineen. In: A. Thienemann (Ed.), Das phytoplankton des Süßwassers. 3 Teil. Schweizenbart, Stuttgart. 310 pp.

Pascher A. (1939): Heterokonten. In: L. Rabenhorst (Ed.), Kryptogamen-Flora von Deutschland, Österreich und der Schweiz. Bd. 11. Akad. Verlag., Leipzig. 1092 pp.

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Baker A.F., Bold H.C. (1970): Phycological Studies X. Taxonomic studies in the *Oscillatoriaceae*. Univ. Texas Publ., 7004. 73 pp.

Brambilla E., Hippe H., Hagelstein A., Tindall B.J., Stackebrandt E. (2001): 16S rDNA diversity of cultured and uncultured prokaryotes of a mat sample from Lake Fryxell, McMurdo Dry Valleys, Antarctica. Extremophiles, 5: 23-33.

Broady P.A. (1982): New records of Chlorophycean micro-Algae cultured from Antarctic terrestrial habitats. Nova Hedwigia, 36: 445-484.

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11 Fauna: Freshwater Micro-invertebrates

(Cynan Ellis-Evans, Eugenio Rico)

Antarctic freshwaters contain a range of micro-invertebrates. Where microbial mats occur, Rotifera, Tardigrada, Nematoda and sometimes Gastrotrichia, Turbellaria and Enchytraeidae may also be found (the last two are not reported from continental freshwaters). Nekto-benthic Crustacea (calanoid copepods, cladocerans and ostracods) are common in some areas. Harpacticoid copepods have been reported from both coastal continental and sub-Antarctic lakes. In the maritime and sub-Antarctic regions the anostracan, *Branchinecta gainii*, is frequently encountered whilst on sub-Antarctic South Georgia, the diving beetle *Lancetes angusticollis* (Coleoptera) is a substantial predator. One example of an Antarctic water mite (Hydracarina) has been found in a Signy Island pool and two types of chironomid midge occur in freshwaters of the South Shetland Islands and the northern Antarctic Peninsula islands. More Diptera occur in the sub-Antarctic but are yet to be fully documented. Sub-Antarctic freshwaters are long overdue a comprehensive investigation of micro-faunal diversity.

Sampling of tardigrades/rotifers/nematodes/ostracods/gastrotrichs – planktonic sampling

Rotifers and gastrotrichs (and ostracods in shallow water bodies) are the only groups that are regularly found in the water column but a fair amount of resuspension and catchment influx faunal contributions also get into the water column. Numbers are usually low so several litres of water are routinely required per sample. However in spring, maritime lakes can contain enormous numbers of juvenile stages of copepods and anostracans.

Water can be sampled using a large sampling bottle (5L) or a simple diaphragm pump to draw water from different depths. If using a diaphragm pump ensure that pumping is done slowly to minimize shear stresses for micro-fauna. Both systems will work through an ice hole. Known volumes can then be passed through a large filter chamber (7-10 cm wide, 30 cm long) with fine mesh (40-50 µm) windows extending 10-15 cm long and 5 cm wide down the sides of the chamber, through which the water passes whilst the planktonic invertebrates are concentrated in the bottom of the chamber (shaped as an inverted cone) below the mesh sides. Concentrated samples can then be drawn off into a small sampling bottle through a simple valve opening at the base of the chamber. The filter chamber has proved very effective year round, though in winter filtration may need to be done in either a field hut or in a small tent to minimize the risk of freezing. This system can also be used for planktonic Crustacea (see below). Store samples in an insulated container to prevent freezing of samples during return to the laboratory.

An alternative approach is to use artificial substrates such as polyurethane blocks or glass slides placed into cork bungs, both of which have been used successfully in the Dry Valley lakes and at Signy Island respectively. The glass slides can be either cleaned on one side and examined directly by microscopy or a fine brush used with tap water to drag the attached material into a vial from where subsamples can then be taken for microscopical examination.

Planktonic sampling of Crustacea

Copepods and, to a lesser extent, cladocerans have excellent "escape" responses that can carry them away from pump heads or coring devices but if large volumes of water are being filtered the pump system nevertheless seems to obtain reasonable data. Large sampling bottles (~ 5L) with automatic closure seem to collect effectively and vertical net hauls (some with automatic closure) can be simple and reliable. If nets are used they should have a mesh size of 60 µm. All these methods can be used through an ice hole.

Planktonic sampling of Anostraca

Only the one representative in Antarctica

(*Branchinecta gaini*) and it does not occur in continental Antarctica. Essentially nektobenthic, its larval stages can appear in vast numbers in spring (often under ice cover) and adopt a planktonic habit. Anostracans are relatively tolerant of sampling by diaphragm pumping and can also be netted easily.

Benthic sampling
Most micro-invertebrates are found associated with benthic material. Collecting micro-invertebrates from freshwater lakes and pools can be done by core, dredge or grab for deeper water samples but most commonly coring methods are used, being simpler, lighter and more flexible for operating from a boat or through an ice hole. Sectioning or sub-coring of sediment cores provides for quantitative or semi-quantitative extraction.

Freeze corers
Freeze corers (using dry carbon dioxide carried to the site in small pressurized cylinders rather than liquid nitrogen) have proved effective in Antarctic work when comparing populations at various depths in sediments, particularly with sandy sediments, which are difficult to core by standard methods. The corer is simply a weighted hollow tube filled with dry ice and shaped to a point at the bottom to aid sediment penetration. Sediments freeze to the outside of the tube. However, where the sediments are silty, the majority of benthic invertebrates are restricted to the top 1 cm, which is where greatest physical disruption often occurs with this method so great care needs to be taken in interpretation. The method is also not recommended where consolidated microbial mats overlie the sediments as these corers drag the mats down the sediment profile.

An alternative method, used successfully at Signy Island for studying micro-invertebrates within microbial mats, involves cutting pieces of microbial mat and transferring it, still under water, to a petri dish, which is then covered with a petri lid. This method can be used in shallow waters or by SCUBA divers in deeper water. Small subcores can be cut with a cork borer for quantitative analysis. Once returned to the lake or pool surface the petri dish can alternatively be placed into a wide-mouthed thermos containing solid carbon dioxide. This freezes the mat within seconds and the sample can then be returned frozen to the laboratory and stored until analyzed. Using freeze-fracture and scanning electron microscopy or inverted microscopy with a cold stage, detailed profiling of the mat structure is possible and has been employed to examine benthic diatom attachment strategies but was incidentally shown to work well for micro-invertebrates.

For shallow water sediment sites
a simple siphon technique using a 60 ml catheter syringe and an 18 cm length of ~9mm bore semi-rigid plastic tubing can be very effective. With care this allows 1-20 ml samples of surface sediment and unconsolidated microbial mat to be sampled without dilution by underlying sediments. The same technique has been used by SCUBA divers in deeper water. This methodology is semi-quantitative and most effective for silty sediments.

Artificial substrates
in wire baskets placed in the water body for extended periods of time (30-60 days) have also been used successfully.

An inverted glass bottle with a filter funnel inserted through the neck of the bottle (same principle as a lobster pot) and moored immediately above lake microbial mats has been found to be a very effective in trapping large numbers of copepods and (to a lesser extent) cladocerans over a 24-48 hr period. However if there is also a copepod predator present (e.g. *Parabroteas sarsi* in maritime freshwaters) and this gets into the trap, very substantial predation results so traps should not be left for more than 24 hr.

Two species of Dipteran midges (Chironomidae) occur in the maritime Antarctic (*Belgica antarctica* and *Parochlus steinii*) and these have freshwater larval stages. They are generally found under stones in streams, or amongst microbial mat and small stones in pools and lake shallows rather than in the organic sediments they are associated with elsewhere. Samples can be collected with a hand net (20 µm mesh size ideally but certainly 45µm) or the syringe sampler described above.

Extraction of benthic micro-invertebrates
It is possible to sort samples by hand under a dissecting microscope but micro-invertebrates are often closely associated with benthic material and so this approach is slow and has low extraction efficiency. In sandy sediments, qualitative sampling can be made by stirring collected sand in a container of water and decanting immediately after the sand settles. The decanted water containing micro-invertebrates is passed through a sieve with ~ 45 µm mesh size and the specimens rinsed from the screen.

A popular variant of this method that has proved relatively efficient for the small forms that dominate Antarctic sediments is elutriation/decantation where water is passed rapidly up through sediments and the invertebrates being less dense than sand or silt are passed out of the container and retained in fine sieves (20 or 45µm mesh). This method is not so effective where microbial mats are present, as the animals are bound up in the mat structure.

A variety of density centrifugation methods have been used successfully to extract micro-invertebrates. Water and sucrose have both been employed but particular success has been found with Ludox AM (Du Pont product, colloidal silica),which has a relatively low toxicity, and, unlike sucrose, negligible osmotic influence. Other similar compounds (e.g. Percoll) are available from the biochemistry sector but these are prohibitively expensive. A suspension of the sample of sediment or vegetation is filtered through stacked sieves of 500µm and 44µm. If mats are present, better release of tardigrades in particular can be induced by putting the sample first through a Waring homogenizer for 1 minute (though this can reduce counts of rotifers) and then sieving. The homogenization step breaks up the mat structure. The sediment remaining on the fine mesh sieve is centrifuged with water at about 3000 rpm for 5 mins. The supernatant is poured through the fine mesh sieve and the remaining sediment is mixed with Ludox and centrifuged at about 5000 rpm for 1 min. The supernatant is immediately rinsed through the fine mesh sieve with water and the residue fixed or washed into a petri dish for examination under a dissecting or inverted microscope. There may be a need to repeat the Ludox stage up to three times to ensure the most complete extraction, particularly for nematodes.

Fixation of micro-fauna
Tardigrades can be fixed via boiling ethanol (85%+) added to small volumes of water or with Ripart & Petit fixative: 75ml of camphor water (as fungicide) , 75 ml of distilled water, 1ml of glacial acetic acid, 0.3g of cupric acetate and 0.3g of cupric chloride. As an alternative to camphor water, one can add 75ml of distilled water with 3-4 drops of 40% formaldehyde. Initially there is contraction but this reverses after 1-2 hours in the fixative. In large volumes of water, the addition of 5% buffered formalin or gluteraldehyde will preserve tardigrades, but may cause contraction, which then makes animals more difficult to identify.

Sediment samples containing nematodes can be fixed by addition of 10% formalin after collection. Nematodes often adhere to particles and several rinses with water improves extraction efficiency. Addition of Rose Bengal helps to identify fixed nematodes. For quantification of fresh samples pre-treatment with $MgCl_2$ relaxes both nematode and rotifer specimens.

Rotifers
are particularly prone to contracting into unidentifiable lumps if there is not proper anesthetization before fixation. Illoricate rotifers should always be identified alive to be able to see critical diagnostic characters. $MgCl_2$ has been frequently employed as a relaxant. Loricate rotifers can be fixed without anesthetization and common fixatives are 2-10% neutralized formalin, 4-5% Lugols Iodine or 30-70% ethanol. Dyes such as cresyl blue, rose Bengal or neutral red (~0.1%w/v) can be added to aid sorting of fixed samples.

Fixation of Copepods, Cladocerans, Ostracods & Anostracans
Animals can be preserved in the field with 70% alcohol. Copepods and ostracods can also be preserved with 5-10% formalin but ostracod carapaces will start to decalcify after a few months. Cladocerans can be preserved with 3-

5% formalin with 40g l^{-1} sucrose to minimize body distortion and prevent egg loss from pouches. 4-10% formalin can be used for anostracans but there is a tendency for hardening of body structures. Again the use of a relaxant such as $MgCl_2$ prior to fixation improves extraction efficiency in copepods, cladocerans and anostracans. After sorting, specimens should be transferred to 70% ethyl alcohol (copepods, ostracods, anostracans) with 10% additional glycerin (cladocerans). Dissection is needed to identify to genus or species in copepods, ostracods and anostracans. Female specimens are preferred for harpacticoid identifications though males are required for identifying Antarctic genera of certain copepod families such as the Canthocamptidae.

Shallow water sampling strategies
It is frequently the case in sampling Antarctic freshwater shallows that micro-invertebrate distribution is markedly heterogeneous. This is particularly the case in streams and open lake/pool systems and less so in closed systems (no outflows). Where through flow occurs a single sample from a shallow water body is not recommended. If it is unavoidable, we recommend sampling close to the outflow point of the lake/pool or, for streams, in the vicinity of microbial mats if present.

Although closed systems tend to have more broadly homogeneous distributions, various factors, such as katabatic winds driving material to the downwind side of the water body, will influence distribution so multiple samples are still recommended. In maritime Antarctic lakes and pools samples are always taken at the outflow point (if present) and at regular intervals (at least four more locations) around the entire shoreline. In streams several samples should ideally be taken at regular intervals across the stream at each sampling station and certainly at several points along the stream length, particularly where the percentage of ice/snow to exposed rock and vegetation is changing along the streams length.

12 Paleolimnology

The recent history of lakes and their watershed can be investigated using lake sediment cores. Remains from different organisms are accumulated in the sediment of the lakes. In many cases these can be used as bioindicators of climatic or watershed variations. Holocene climate and environment can be inferred from the interpretation of lake sediments. Different geological, biological and chemical deposits can be used in these inferences, although in this section we will deal only with the biologically originated deposits.

12.1 SUB-FOSSIL DIATOM COMMUNITIES

(Manuel Toro)

The objective is to study possible environmental changes in lakes and their catchments, by means of the analysis of the subfossil diatom communities in sediment cores. At least two sediment cores have to be taken at the deepest point of the lake with a gravity type corer (modified Kajak corer) following Wetzel & Likens (1979). The corer must be lowered to approximately 1 m above the sediment surface and then fall freely into the sediments. After slowly retrieving the corer, place a rubber stopper into the botton opening before removing it from the water to prevent loss of the sample. In the boat, stopper the upper end and store the corer erect. After describing the sediment stratigraphy, the sediment core should be extruded on site by means of an extruding device (Uwitec system) and slices should be cut on site. These sediment slices (2-3 mm slices for the top ten cm of the core; 5 mm slices for the rest of the core) must be preserved in sterile plastic bags in the dark at 1-5 °C. In the laboratory the slices can be analysed for dry weight (105 °C for 24 hours or until a constant weight is obtained), loss-on-ignition (550 °C for 2 hours), grain size, chemistry and diatom communities (see diatoms section). Possible changes in diatom species composition and abundance along the sediment cores can be studied and discussed in relation with environmental changes in the recent past.

12.2 ISOLATION OF FAUNAL REMAINS FROM SEDIMENT SAMPLES

(L. Cromer, J. Gibson)

The development of the faunal communities currently found in Antarctic lakes can be traced by studying remains, e.g. exoskeletons and other body parts, eggs, spermatophores and faecal pellets, preserved in the lake sediments. Two major routes to development of the communities can be identified. In saline lakes that were formed by the isolation of pockets of seawater during post-glacial isostatic rebound, the initial limnetic community was defined by the organisms present in the lake when it was finally cut off from the larger marine ecosystem. Although it is possible that other species have invaded the lakes subsequent to the isolation event, the general trend appears to be a decrease in both biodiversity and abundance with time as the lakes become more oligotrophic. In freshwater lakes the situation is different, as there is no pre-existing inoculum when the lake was formed. Therefore the organisms present in the lake have to have invaded from elsewhere, setting up viable populations within the lake. Studying the distribution of remains of these organisms in the sediment cores provides an indication of such processes.

Antarctic lakes are particularly amenable to palaeofaunal studies, as they in general do not contain bottom grazers that recycle organic material (rendering it unidentifiable) that accumulates at the base of the water column. Chironomids do occur in lakes in the Antarctic Peninsula and maritime Antarctic zones, which probably preclude such studies in these lakes. However, remains of the chironomids themselves will be present in the sediments from these lakes, and these remains themselves are important indicators of lake environmental change, and, more generally, climate change.

Unlike sedimentary studies conducted on siliceous diatoms and pollen, where sediments

can undergo acid treatment to remove organic matter, studies using animal remains need to retain organic matter. For sediments that are highly organic, it is difficult to isolate faunal remains from the strongly clumped microbial mat and other detritus. A number of different methods for the isolation of faunal remains from sediments has been developed, including centrifugation and differential sedimentation. These methods are not applicable to fine-grained, organic rich sediments typically found in Antarctic lakes. The use of polyphosphate compounds (e.g., the commercially available water-softener Calgon®, sodium hexametaphosphate) as a deflocculating agent for organic rich sediments allows isolation of faunal remains in good condition, and this has become the method of choice in our studies of Antarctic lakes.

Up to four grams of sediment are placed in a small vial. A 20% w/v solution of Calgon® is poured over the sediment until it is immersed. The sample is then agitated gently so that no large fragments remain. A few drops of a solution of Rose Bengal are added. Rose Bengal is a stain used to highlight organic matter that is rich in lipids. The vial is covered and stored at a 4 °C overnight (or longer if necessary), enabling complete deflocculation of the organic matter to occur and the Rose Bengal to penetrate the faunal remains.

The samples are then washed through a set of 200, 100 and 44 μm stacked sieves with GF/F filtered water of similar salinity to the sample (note: use of unfiltered water, even treated tap water, in this step can introduce contamination). Other choices of sieve size can be made depending on availability and the characteristics of the particular remains of interest. Each of the sieves is then back-washed into a separate, labeled jar. Each sediment fraction is then transferred to a Bogorov or similar tray for counting and identification. The size, shape, colour and any morphological markings of faunal remains are recorded. Identification is often by comparison to organisms extant in the lakes, or, if modern material is not available, to standard texts. Photos of all remain types should be taken to develop a library of images that will aid in identification. Due to the large amount of organic detritus in the lake sediments, the finest sieve sample often contains too much material to be processed efficiently in a reasonable time. A plankton splitter can be used to subsample this fraction.

The data obtained are on a wet weight basis. To convert to number g^{-1} dry weight, a separate subsample of the sediment sample should be dried, and a conversion factor calculated.

13 Other habitats

13.1 MEROMICTIC LAKES

(John Gibson)

Meromictic lakes are those lakes in which the complete vertical mixing of the water column does not occur at any stage of the year. Meromictic lakes are particularly common and widespread in Antarctica, with such lakes known from McMurdo Dry Valleys, Vestfold Hills, Windmill Islands, Bunger Hills, Syowa Oasis, Deception Island, and probably other areas. In most, if not all, Antarctic meromictic lakes the resistance to mixing results from an increase, often marked, in salinity, and therefore density, with depth. The stabilising salinity profiles are often counteracted by a destabilising temperature profile, in which temperature increases with depth. However, the density decrease that results from the thermal characteristics does not outweigh the effect of increasing salinity. Horizontal mixing in these lakes appears to be quite efficient.

Antarctic meromictic lakes are characterised by sharp thermo-, halo- and pycnoclines. Anoxia is often associated with a pycnocline. In some lakes, the structure is simple, with a single pycnocline separating the upper, mixed, mixolimnion from the lower, stagnant, monimolimnion. Other lakes are more complex, with series of pycnoclines often separating mixed layers (e.g. Lake Vanda, McMurdo Dry Valleys). In most cases, the water that does not undergo vertical mixing is anoxic and nutrient rich. These characteristics need to be taken into account when sampling meromictic lakes.

13.1.1 Physical Structure Determination

Due to the inherent structural features of the water columns of meromictic lakes, it is important that the depths from which any data, e.g., salinity or temperature, are obtained are stated explicitly. Most meromictic lakes have closed basins, and therefore the water level in the lakes can rise or fall depending on local water balance. Depths of (semi)-permanent features, such as pycnoclines, can appear to move within the lake when measured from the lake surface. However, this is the result purely of changing water level and zero reference level, and in absolute terms (i.e height with respect to sea level or some other fixed point), the feature remains in the same place.

The zero mark for sampling meromictic lakes should be taken as the equilibrium level of the water surface. If the lake is ice-free, this is trivial. In ice-covered lakes, the zero level should be taken as the water level in the hole drilled through the ice to access the water column. As the ice in effect floats on the water surface (disregarding the support provided at the margins), the water level in the ice hole should reflect the level of the lake if all the ice were to melt. Some studies have used the bottom of the ice as the zero level; this approach has a number of problems, including the variation in the zero level as ice thickness increases or decreases, and the difficulty in measuring its position accurately and using it as the zero for subsequent sampling. Other studies have used the surface of this ice, but this again is a function of ice thickness. These approaches can lead to apparent changes in the position of structural features over a relatively short periods due to a change in the reference level, when in fact they remain fixed in position.

To enable comparisons of a lake over a longer time period (years to tens of years), it is important that the absolute height of the water level is determined by surveying. All meromictic lakes in the Vestfold Hills have a surveying benchmark located nearby that allows accurate determination of the surface level of the lake. If conclusions are to be drawn about changes in lake structures over time, the absolute position of any water column features must be known. An alternative approach is to relate the position of the features to the bottom of the lake. However, it can be difficult to accurately determine the position of the

deepest point in the lake, and this approach is generally impractical. This may be circumvented by use of accurate position markers (e.g. pairs of marked rocks orthogonal to each other that can be lined up so sampling can be undertaken at exactly the same position as previously).

The physical structure of meromictic lakes, with sharp and significant changes occurring over small distances, means that physical profiling of the lakes should be undertaken at fine vertical intervals. Certainly taking readings every metre could result in aspects of the structure being missed. Profiles are best collected with a submersible data logger – units are available that record not only temperature and conductivity (needed for basic understanding of the physical structure), but also dissolved oxygen, pH, chlorophyll etc (see the water column structure section). Two approaches to collecting profiles are possible. The first is to allow the data logger to sink slowly through the water column. Some buoyancy may have to be added to reduce the rate of sinking. Fast data collection rates, of the order of a sample per second, allow very detailed pictures of lake structure to be developed. This approach has a problem in that these data logger units measure depth via the pressure exerted by the overlying water column. In general they are calibrated to freshwater or seawater. The problem in meromictic lakes is that salinity changes throughout the water column, sometimes markedly. Therefore a single conversion from pressure to depth will misrepresent true depth. For low salinity lakes it is possible to correct this knowing the salinity and temperature (and therefore density) of the overlying water, though this can be a non-trivial calculation. For high salinity lakes, where the relationship between density, temperature and salinity is poorly known, this becomes difficult or impossible. In this case a different approach can be used. The profiler is attached to a line that has a mark every 10 cm (or other depth interval as required). The profiler is then lowered to each mark sequentially. It is left at each depth for a period of time to enable thermal equilibrium to be obtained, and also to make it clear in the data when the profiler was dropped to a new depth. The profiler is set up to measure every few seconds, and the data immediately prior to the unit being lowered by another increment is used when plotting the profile. This method is tedious, but provides data at accurately measured depths.

13.1.2 Chemical and Biological Sampling

Knowledge of the structure of the meromictic lake should be taken into account when designing a sampling routine. Unless there is good reason to do so, it is probably advisable not to sample directly at a sharp pycnocline, as there may be marked differences in the characteristics of water at the top and bottom of a water sampling bottle.

Care should be taken to accurately define what depths samples were collected from. As discussed above, the zero level used for sampling should be surface level of water in the lake. If a sample is taken from, for example, 6 m, it should be made clear what part of the sampling bottle is at 6 m when the sample is collected. It is recommended that this is the middle of the sampling bottle. Better still is to report the range which the sample covered. If the sample was taken with a Niskin or similar bottle 0.5 m in length, the ‘6 m’ sample becomes an integrated sample from between 5.75 m and 6.25 m. How sampling depths are treated should be stated explicitly in the methods sections of any publication.

After collection it is necessary to make sure that the water sample is well mixed. This is best done by inverting the water sampler a number of times. If dissolved oxygen, pH or similar variables are not being measured, the sample can be transferred to a secondary container in which the sample can be more easily homogenised. If the sample is not mixed, the differences in characteristics between the top and bottom of the sample may lead to false data. For example, if the oxic-anoxic interface is included in the sample, dissolved oxygen concentrations in samples taken from the bottom of the bottle will be zero, and sulfide concentrations in samples taken from the top of the bottle also zero. This could seriously misrepresent the actual profile.

It is preferable to sample stratified systems at a defined depths, i.e. not over a depth range

implicit in the use of a sampling bottle. At least two approaches can be used to achieve this goal. Peristaltic pumps can be used to collect water from a particular depth. In order to make sure that water flows to the pump intake as far as possible in a lateral fashion, the pumping rate should be as low as practicable. Increasing flow rate will increase the tendency for water to be sampled from above and below the inlet. The inlet tube to the pump is marked prior to sample collection so that particular depths can be sampled, and the line flushed with water from any previous sampling prior to collection of new samples. I have also used a simple 60 ml syringe attached to a three-way valve and a length of Teflon tubing to collect a detailed set of samples from a hypersaline meromictic lake. In this case the end of the tubing was weighted and lowered to the required depth. The syringe was slowly filled a number of times, and the water expelled to waste. After it was deemed that water from the previous sampling depth had been completely flushed from the system, the sample was transferred to a plastic bottle. This method is, however, time consuming and limited to relatively shallow lakes.

Meromictic lakes usually are anoxic in their monimolimnion. Nutrient concentrations in this zone are typically far higher than in the surface waters. In particular, soluble reactive phosphate can be hundreds of times higher, and the solubility of metals (e.g. iron and manganese) in oxygen-free conditions is far higher than in oxygenated waters. Disposal of anoxic water at the surface of the lake or on the surface of the ice can lead to short-term localised increases in nutrient concentration that do not reflect typical conditions in the lake. Productivity may increase as a result of this input. Therefore any excess lake water collected from throughout the water column during sampling should be collected in a waste container, and disposed of into the marine ecosystem where it will be rapidly diluted.

The high concentrations of nutrients in the anoxic waters of meromictic lakes may make it necessary to adapt analytical procedures. The dilution of anoxic water with freshwater of known nutrient concentration prior to analysis may assist in such cases.

13.2 WET TERRESTRIAL ENVIRONMENTS

(*Antonio Quesada*)

In addition to lakes and streams, wet terrestrial environments constitute important limnetic environments, and in many cases cover larger surface areas than lakes and streams together. Wet terrestrial ecosystems are typically the result of snow/ice melt, or permafrost's active layer thawing and consist of water saturated soils. Commonly these areas are waterlogged every year, and they form intermittently wet ecosystems, depending on environmental conditions such as precipitation, incident radiation, temperature, etc.

The organisms inhabiting these intermittent ecosystems are particularly interesting, since they have to be particularly well adapted to environmental changes. In the same day they can be frozen solid as well as reach high temperatures (14-16º c) because of the direct radiation effect. Cyanobacterial mats are typically the dominant biomass in these ecosystems, but ciliates, rotifers and other invertebrates can be present as well, depending on the latitude.

Sampling these ecosystems is relatively easy, and many of the techniques related in the previous sections can be applied to wet terrestrial systems. However, particular care has to be taken when sampling organisms, since within the same wet area, several different microbial mats can be found, showing different biodiversity and functionality. Typically the colours (from pale orange to intense purple) and cohesiveness indicate different communities.

The chemical characteristics of the water overlying the microbial mats are measured in the same way as described before, but the water has to be collected by syringe, sucking up the water very gently. The water samples

have to be filtered through GFF filters and stored as related in the relevant section of this manual. The chemical conditions to which the organisms within the microbial mats are exposed are quite different to those of the overlying water, and well marked chemical gradients exist within the microbial mat structure. The use of microelectrodes may give a better idea of the chemical environment surrounding the organisms (see microelectrodes section). However, it is advisable to make the micro-profiles immediately after coring the mat, since chemical characteristics will change rapidly, and in many occasions the position of the organisms will also change within hours of collection. It is also important to minimize environmental variations with respect to natural conditions, temperature, radiation, etc. while the micro-profile is taken. Otherwise the obtained profiles will be artefactual. The water within the microbial mat can also be collected by extremely gently pressing the mat on a funnel, thus collecting the pore water. Typically the chemical characteristics obtained in this way will differ very much of those found in the overlying water. Both measurements are interesting and may help explaining different aspects of the ecology and physiology of the microbial mats.

14 Molecular taxonomy analyses

14.1 FLUORESCENCE IN-SITU HYBRIDISATION- FISH

(Cynan Ellis-Evans)

This is a very new and powerful tool for probing microbial community structure in *situ*. Probes are raised to select at different taxonomic levels - so a probe might target a domain such as Bacteria, a sub-class such as α-Proteobacteria, a species or a genus. When the probe is added to a water sample it will become associated with its target organism and all cells of that organism will in theory fluoresce. It is possible to add several probes which fluoresce at different wavelengths and detect the different target cells with a flow cytometer or by cycling through different selective wavelength filters on a fluorescence microscope. In reality most researchers are finding roughly 50% efficiency in tagging cells though certain labs have now improved the tag rate to >80%. This will become a major tool in microbial ecology over this decade and provide the first detail of microbial community structure dynamics in natural systems. The method outlined below is targeting only domain and subclasses but has been used successfully at Signy Island.

- 10 ml of each lake water sample is filtered through a black polycarbonate 0.2 µm screen membrane filter (Poretics, Livermore, CA, USA).
- Cells are fixed with 2 ml of 4% para-formaldehyde in phosphate buffered saline for 30 minutes. A gentle vacuum is then applied and cells rinsed, initially in 5 ml phosphate-buffered saline, then in 5 ml distilled water.
- Filters are removed from the filtration apparatus, air dried, placed on a glass microscope slide and stored at -20 °C. All preservation and hybridization conditions were selected to minimize impact on the integrity and characteristics of cells.
- 16 µl of hybridization buffer containing one of six different *in situ* hybridization probes is added to each filter and incubated at 46 °C for 90 minutes in a closed hybridization tank. The following oligonucleotide probes were used at Signy Island:

i. EUB 338, complementary to a region of the 16S rRNA (338-355) specific for the domain Bacteria (5'-GCTGCCTCCCGTAGGAGT-3');
ii. ALF1b, complementary to a region of the 16S rRNA (19-35) conserved in the alpha-subclass of *Proteobacteria* and some other *Proteobacteria* (5'-CGTTCGYTCTGAGCCAG-3');
iii. BET 42a, complementary to a region of the 23S rRNA (1027-1043) specific for the β-*Proteobacteria* (5'-GCCTTCCCACTTCGTTT-3');
iv. GAM 42a, complementary to a region of the 23S rRNA (1027-1043) conserved in the gamma-subclass of *Proteobacteria* (5'-GCCTTCCCACATCGTTT-3');
v. CF 319a, complementary to a region of the 16S rRNA (319-336) conserved in the *Cytophaga-Flavobacterium* group (5'-TGGTCCGTGTCTCAGTAC-3').
vi. ARCH915, complementary to a region of the 16S rRNA (915-934) conserved in the Archaea (5'-GTGCTCCCCCGCCAATTCCT-3').

- Each probe is covalently linked at the 5'- end to a single fluorescent dye molecule – in this case the very photostable indocarbocyanine dye CY3.
- The atmosphere in the hybridization tank is saturated with excess hybridization buffer on filter papers at the base of the tank.
- Each slide is then rinsed with 20 ml pre-warmed washing buffer at 48 °C over a period of 15 minutes.
- Six hybridization buffers are prepared with 0.9 M NaCl, 20 mM Tris-HCl (pH7.4), 0.01% SDS and 50 ng.ml^{-1} of one of the probes.
- Formamide is added to the following probes: ALF 1b, BET 42a, GAM 42a and CF 319a in concentrations of 20, 35, 35 and 15 % respectively. Formamide is used to increase the permeability of the cells or the availability of the target sites.
- Six washing buffers are also prepared with 20 mM Tris-HCl (pH7.4), 5 mM EDTA,

0.01% SDS and NaCl in the following concentrations: 0.9M EUB; 0.225M ALF 1b, 80 mM BET 42a, 80 mM GAM 42a and 80 mM CF 319a. The washing buffer contains concentrations of NaCl, which correspond to the formamide concentrations in the hybridization buffer. The stringency of the washing step can be adjusted by lowering the sodium chloride concentration.

- The filters are placed on slides, air-dried, mounted with the glycerol containing mountant Vectashield (Vector Laboratories Ltd, Peterborough, UK) to minimize bleaching and viewed at x 1250, under oil immersion, with a Leitz Labalux epifluorescence microscope equipped with a 50W mercury lamp and a CY3 filter set (filter set 41007A, Chroma, USA).
- Parallel counts using DAPI provide a total cell count from which the probe counts as a percentage of total counts can be established.

14.2 PROTOCOLS FOR STUDIES OF CYANOBACTERIAL MOLECULAR DIVERSITY

(A. Taton, S. Grumisic, Annick Wilmotte)

Extraction of DNA

The DNA extraction protocol described here is derived from Engelen (PhD thesis), itself deriving from Smalla et al (1993). 0.5g mat samples were suspended in 0.5 ml of SNT-Solution (500 mM Tris HCl, 100 mM NaCl, 25% saccharose, sterilized), supplemented with 26 µl freshly added lysozyme (25% (wt/vol)). The resulting suspensions were shaken and incubated for 30 min at 37°C. After incubation 0.5ml of Solution II (500 mM Trisbase, 500 mM EDTA, 1 % SDS, 6% phenol), and 0.25g glassbeads (0.17-0.18 mm, Braun Biotech) were added to the sample and shaken for 60 sec in a Bead-beater (Braun Biotech). The resulting suspension was placed on ice for one hour and vortexed every 10 min. After incubation, the suspensions were centrifugated for 10 min at 3000 rpm (MinifugeT, Heraeus) and 1 ml of the aqueous phases were mixed with an equal volume of phenol, after which they were centrifuged for 5 min at 13000 rpm. The supernatants were then transferred to new tubes and extracted with equal volumes of phenol\chloroform\isoamylalcohol (25:24:1), and re-extracted with equal volumes of Chloroform/Isoamylalcohol (24:1). A standard Na-acetate-ethanol precipitation followed and dried pellets were resuspended in TE buffer (10 mM Tris-Cl, 1 mM EDTA (pH 8)), giving a final volume of 100 µl.

For the clone libraries,

the crude DNA preparations were subjected to several cesium chloride (CsCl) and potassium acetate (KAc) based purifications steps, following a modified version of the protocol of Smalla et al (1993). 200 mg $CsCl_2$ were added, mixed and centrifuged 10 min at 14000 rpm, rt after a 2h incubation at 20°C (mix every 30 min). 3 volumes of water and 0.6 volumes of 80% isopropanol were added to the supernatant and centrifuged for 15 min at 14000 rpm, rt after a 30 min incubation at 20°C. Subsequently the supernatant was discarded and the pellet dried. The DNA was dissolved by adding 100µl of 1xTE, followed by 20µl of 8M Kac and 1h30 incubation at 20°C. After 15 min centrifugation at 14000 rpm, rt, 0.6 volumes of 80% Isopropanol were added to the supernatant, mixed and incubated 30 min at 20°C. After centrifugation for 15 min at 14000 rpm and 4°C, the supernatant is discarded, and the pellet dried. The DNA is dissolved in 200µl 1xTE.

For the DGGE,

the crude DNA preparations were subjected to purifications steps using the commercial kit: Wizard DNA clean-up system (Promega, USA), following the manufacturer' s instruction.

TABLE 1. *Primer sequences, target sites and references*

Primer[a]	Sequence 5'→ 3'	Target site[c]	Reference
16S27F	AGA GTT TGA TCC TGG CTC AG	7-27	Wilmotte et al. 1993
16S378F	GGG GAA TTT TCC GCA ATG GG	359-378	Nübel et al. 1997
16S781R(a)[b]	GAC TAC TGG GGT ATC TAA TCC CAT T	781-805	Nübel et al. 1997
16S781R(b)[b]	GAC TAC AGG GGT ATC TAA TCC CTT T	781-805	Nübel et al. 1997
16S784R	GGA CTA CWG GGG TAT CTA ATC CC[d]	784-806	Derived from Nübel et al. 1997
16S1494R	GTA CGG CTA CCT TGT TAC GAC	1494-1514	Wilmotte et al. 2002
23S30R	CTT CGC CTC TGT GTG CCT AGG T	30-52	Lepère et al. 2000

[a] R (reverse) and F (forward) designations refer to the primer orientation in relation to the rRNA.
[b] A 38-nucleotide GC-rich sequence (5' -CGC CCG CCG CGC CCC GCG CCC GTC CCG CCG CCC CCG CC-3') is attached to the 5' end of the reverse primers.
[c] *E. coli* numbering of 16S rRNA or 23SrRNA nucleotides.
[d] W, a A/T nucleotide degeneracy.

DGGE

a) PCR amplification of 16S rDNA fragments and DGGE analysis

The 422 bp long 16S rDNA fragments were generated by semi-nested PCR. The primer pair for the first PCR was 16S378F and 23S30R. This PCR was performed in 50µl (total volume) reaction mixture, containing 0.5 µl of mat DNA, 1XPCR buffer of Super Taq Plus, 0,2 mM dNTPs, 0,5 µM primer 16S27F, 0,5 µM primer 23S30R, 1 mg ml^{-1} BSA (Sigma, USA), 1U Super Taq Plus polymerase with a proof reading activity (HT Biotechnology, UK). Thirty cycles of amplification were carried out with the Gene Cycler (Bio-Rad, USA) as follow: 1 incubation of 5 min at 94°C, 30 cycles of 45 s at 94°C, 45 s at 54°C, 2 min at 68°C and a final elongation step of 7 min at 68°C. 0.5 µl of the resulting PCR products served as template for the second PCR reaction, which was performed with the primers 16S378F and the primer 16S781R (a) and (b). A 38-nucleotide GC-rich sequence is attached to the 5' end of the separate reverse primers. The reaction conditions were the same as described above except for the 35 cycles of amplification. They were carried out as follow: 1 incubation of 5 min at 94°C, 35 cycles of 1 min at 94°C, 1 min at 60°C, 1 min at 68°C and a final elongation step of 7 min at 68°C. 2 distinct reactions were realized for each primer pair (16S378F - 16S781R(a) and 16S378F - 16S781R(b)) and the resulting PCR products were maintained separated.

DGGE was carried out in a Decode gene system (Bio-Rad, USA), and performed as described by Muyzer et al. (1996) with the following modifications. The PCR products obtained with the primer 16S781R (a) and (b) were applied separately onto 6% (wt/vol) polyacrylamide gels (acrylamide/N,N'-methylene bisacrylamide ratio, 37:1 (wt/vol)) in 1x TAE buffer (pH 7.4) which had been prepared from sterile solutions and casted between glass plates. The gel contains a linear gradient of 40 to 65 % denaturant (100% denaturant = 7 M urea plus 40% (vol/vol) formamide). Electrophoresis proceeded for 16 h at 45 V and 60°C. Denaturing gradient, voltage and electrophoresis time were determined by perpendicular DGGE experiment. The gel was stained with ethidium bromide and photographed.

The DGGE bands were excised with a surgical scalpel and used for reamplification and sequencing. Each small acrylamide block was

placed in 100 µl of pure sterile water for 2 hours at room temperature. The solution was used as template for PCR amplification as describe above. The PCR products were then rerun in the same conditions as described above to confirm their position relative to the bands from which they were excised and to detect potential heteroduplexes.

Sequencing was carried out by Genome Express (Paris, France) on a ABI PRISM system 377 (PE Applied Biosystems, USA). A partial 16S rDNA sequence of circa 350 bp from the cyano-specific primer 16S378F described by Nubel et al. (1997) was determined for the major bands.

Clone libraries

a) Cloning of the 16S rDNA plus ITS
PCR Amplification of cyanobacterial 16S rDNA plus ITS of mat DNA was performed with the primers 16S27F and 23S30R. The reaction conditions were the same as described above except for the 37 cycles of amplification. They were carried out as follows: 1 cycle of 5 min at 94°C, 10 cycles of 45 s at 94°C, 45 s at 57°C, 2 min at 68°C; 25 cycles of 45 s at 92°C, 45 s at 54°C, 2 min at 68°C and a final elongation step of 7 min at 68°C. PCR products were purified with the kit Quantum Prep® PCR Kleen Spin Column (Bio-Rad, USA). Poly-A extension of 20 min at 72°C with dATP and the Goldstar polymerase (Advanced Biotechnologies, UK) was performed.

Cloning of purified PCR products was done with the Topo TA cloning Kit (Invitrogen BV, NL) following the manufacturer' s instructions, using a vector/insert ratio of 1:4.

White and light blue transformants were purified twice by streaking and then screened directly for insert by performing colony PCR with M13 forward and reverse primers. The same amplification conditions as those describe above were used, except that 0.8 U of Taq polymerase (Promega, USA) was used and the 35 cycles of amplification were carried out as follows: 1 incubation of 10 min at 94°C, 20 cycles of 1 min at 94°C, 1 min at 55°C, 2 min at 68°C; 15 cycles of 1 min at 90°C, 1 min at 55°C, 2 min at 68°C and a final elongation step of 10 min at 68°C.

Vectors from positive transformants were extracted with Quantum Prep® Plasmid Miniprep Kit (Bio-Rad, USA), following the manufacturer' s instructions. The inserted 16S rDNA plus ITS was re-amplified using the primers 16S27F and 23S30R. The amplification conditions were the same as those describe above.

b) Screening of the clones by ARDRA
Digestion, standardized electrophoresis and gel image analysis with GelCompar (Applied Maths, Kortrijk, BE) were performed as previously described (Scheldeman et al. 1999), excepted that MboI and HpaII (Gibco Life Sciences, USA) were used as restriction enzymes.

Sequencing (DGGE bands and clones)
A partial 16S rDNA sequence of circa 350 bp from the cyano-specific primer 16S378F described by Nubel et al. (1997) was determined for every clone that generated 1 unique ARDRA type. If several clones produced the same ARDRA type, 2 clones were selected at random. Complete 16S rDNA sequences were determined on one DNA strand for one representative clone selected at random in each phylotype. The sequencing was carried out with the cyano-specific primers 16S1494R and 16S784R and the consensus sequences obtained with the software AlignIR (LI-COR, USA). In addition, for the clones for which the full length 16S rRNA sequences were determined as well as for the clones from which different ARDRA types but identical partial 16S rDNA sequences were obtained, the full-length ITS sequences were determined using the primer 23S30R.

Analysis of sequence data (DGGE bands and clones)
The sequences were initially analyzed by similarity search using the software BLAST widely available on internet and chimera detection using the software Chimera Check (cgis/ chimera.cgi?su=SSU/).
The sequences determined in this study were included into the database of the ARB software package (Ludwig et al. 1988) available at http://www.mpi-

bremen.de/molecol/arb/index.html and aligned on the basis of primary- and secondary-structure consideration with the cyanobacterial sequences available from GenBank. Similarity matrixes were calculated for aligned sequences determined in this study and their most related sequences available from GenBank. A threshold of 97.5% similarity was used to distinguish sequences belonging to the same phylotype.

A phylogenetic tree was initially constructed on the basis of almost complete sequences. The maximum-likelihood, maximum-parsimony, and neighbor-joining methods were applied using the ARB software for the tree construction. Partial sequences were then integrated in the tree without allowing it to change its topology according to the maximum parsimony criterion. In addition, distance trees were constructed by Neighbor-joining method with the software package TREECON 1.2 (Van De Peer & De Wachter 1994). Bootstraps were performed, involving the construction of 1000 resampled trees.

15 Process studies

15.1 FOOD WEBS

(Antonio Camacho)

15.1.1 Grazing rates

With the help of microspheres the grazing rates and microbial loop can be estimated with plankton samples.

Zooplankton grazing rates: filter enough water through a 30 µm net up to see the zooplankton on the net (in oligotrophic waters tens of litres may be necessary). Resuspend the net content in 100 ml of filtered lake water. Shake vigorously the resuspended zooplankton and split in several (6) glass vials, add 1 ml of the microsphere suspension. The microsphere suspension should contain different sizes and colours of spheres, the recommended ones are:

- 0.79 µm fluorescent.
- 4.8 µm blue
- 9.4 µm red
- 23 µm white

Incubate each vial at the lake temperature during 5, 10, 15, 20, 30 and 60 min, shaking gently the incubation system every 2 min. Finish the incubation fixing the sample with 1.5 ml buffered 40% formaldehyde. Keep the vials in darkness and cold conditions (4°C). At the microscope collect the animals and count the balls inside and their size. Estimate the grazing rates by animal species and size.

15.1.2 Isotope signature

The lacustrine food web can also be studied in lakes and streams, by analysing the isotopic signature of each of the following size fractionated or trophic levels (up to 10):

- Total seston. Filtrate 5-20 litres of water (as much as possible) through GF/F filters (25 mm diameter). Put the filter on a vial and store frozen.
- < 20 µm. Filtrate 200 litres through a 20 µm nytal filter, collect the filtered water and filtrate through a GF/F filter (25 mm diameter). Store both (nytal and GF/F filters) in separate vials. Store frozen.
- The > 20, > 50 and > 150 µm fractions must be obtained by filtering about 500 litres of water through a sieve tower including sieves of the three sizes. Put the material collected by each of the sieves in a separate vial and store frozen.
- Phytobentos. Collect as much material as possible. Put on a vial and store frozen
- Microbial mats. Take a core in a vial and store frozen.
- Macroinvertebrates. Take at least three individuals of each species, put each species in a vial and store frozen.
- Sedimenting seston can be collected with a sedimentation trap consisting of a 60 cm length, 12 cm diameter PVC tube. The content of the sediment trap must be homogenised, then filter as much water as possible through a GF/F glass fibre filter (25 mm diameter). Put the filter in a vial and store frozen. From the rest of the collected water, fill at least two 250 ml bottles, fix one with buffered formaldehyde (4 % final concentration) and keep the second one in the freezer.

All compartments are analyzed for the natural abundance of both ^{13}C and ^{15}N by mass spectrometry of the natural abundance of stable isotopes. The isotopic signature will give very valuable information about the food webs in the investigated ecosystems.

15.2 CARBON CYCLE

15.2.1 Primary production

The biological entrance of matter, such as C, and energy into the system is considered the primary production. Within this context we can include photosynthetic rates as the main entrance of energy in the ecosystem and also chemosynthesis, partially as a re-utilization of previous energy to incorporate more carbon into the system.

15.2.1.1 Photosynthetic and respiration rates

(Cynan Ellis-Evans, Antonio Quesada)

Photosynthesis is the biological conversion of light energy to chemical bond energy that is stored in the form of organic compounds. Photosynthetic organisms use the light energy to, among other activities, transform inorganic C (typically as CO_2 or HCO_3^-) into organic C (as biomolecules carbohydrates, proteins, etc). Most photosynthetic pathways obtain the electrons required for the reduction of the oxydised forms from breaking up water molecules, producing oxygen into the process. In this way photosynthetic rates may be measured by determining the rate of C incorporation, using isotopes, or by determining the oxygen production. Respiration rates can also be measured by the consumption of oxygen and typically both activities are determined simultaneously.

Oxygen Measurements

Overview

For oxygen measurements in sediment cores, microbial mat incubations or water samples one can routinely use couloximetry, polarographic oxygen electrodes or micro-Winkler titrations. However, at low temperatures, standard polarographic oxygen electrodes drift significantly making it difficult to get a reliable stable reading and when working with small changes in oxygen concentration in a laboratory experiment, consumption of oxygen by the electrodes can become significant. They are however the sensor of choice for field measurements of water column oxygen profiles. It is recommended that when used in the field, a stirrer be attached to the probe to accelerate stabilization.

More robust alternatives for oxygen measurements in incubation chambers are micro-Winkler and couloximetry techniques – both of which involve removing subsamples from incubation chambers for analysis. The micro-Winkler technique described in Peck and Uglow (1990) has excellent sensitivity, requires relatively small sample volumes and is cheap on materials and apparatus but reproducibility is very user-dependent and the procedure is somewhat time consuming. Couloximetry requires little training to use, gives rapid results, has high sensitivity and excellent reproducibility but does require some outlay for equipment. The latter has superceded Winkler determinations in a number of polar research labs.

Miniaturized polarographic microelectrodes (tip size <100µm) are now commercially available and are much less susceptible to drift as oxygen consumption rates are very low. However the electrodes are fragile, have a limited lifetime (~1 year) and are somewhat susceptible to stirring effects. Newly developed fibre optic-based optodes are similarly miniaturized, relatively less fragile and have a lifetime of over 1 year, are very stable and not susceptible to stirring. Both these sensor types can be used for high resolution profiling of sediments and microbial mats and can also be used for static measurements in incubation chambers. They are not recommended for water column profiles.

Couloximetry

The couloximeter is a quasi-solid state galvanic (or fuel) cell that can directly reduce oxygen that is in the gas phase. The glass walled cell is lined with an outer carbonaceous

layer, has a core of nickel supporting active cadmium and between these there is an inert fabric impregnated with aqueous potassium hydroxide. A gas stream delivers traces of oxygen to the cell and this is quantitatively converted to hydroxyl while the "fuel" cadmium is oxidized to hydroxide. The consequent release of electrons generates a current output. The outer layer (positive electrode) and the core (negative electrode) have leads connecting via a high precision resistor (to convert current to voltage) to a measuring device (usually a reporting integrator but it can also be a chart recorder or data logger). The output is in the microvolt to millivolt range. The area under the curve gives the concentration of oxygen though peak height can also be used. The response is linear over the operating range for dissolved oxygen in water but high oxygen concentrations saturate the cell and can take a long time to pass through the system. Aim to keep peak height to <90% of full scale deflection and the peak should pass through in <2 mins. The reaction is temperature sensitive and so care must be taken to prevent substantial temperature fluctuations (>5°C) around the instrument during an extended analysis run.

For use with environmental samples dissolved oxygen has to be stripped from the water. A glass desorber unit is placed upstream of the couloximeter and filled with a weak solution of potassium hydroxide through which oxygen-free nitrogen (OFN) is bubbled at a constant rate of ~30 $ml.min^{-1}$ (some people use 40 $ml.min^{-1}$). This OFN supply is controlled by a two stage regulator on the gas cylinder (for stability) and passed through a high capacity oxygen trap to lower the baseline level of trace oxygen in OFN, followed by an indicating oxygen trap (to identify when the high capacity trap needs regenerating) and then through a precision flow regulator to further stabilize gas flow before it enters the desorber. 0.1 – 0.5 ml aliquots of water sample are injected through an injection port near the base of the desorber using a precision glass repeating micro-syringe. The OFN strips oxygen from the sample and transfers it in the gas phase to the couloximeter cell. Calibration of the instrument is done by injecting known volumes of 0% (OFN-flushed water) and 100% (water bubbled with air for one hour) oxygen standards.

There are very few problems with the method but a number of precautions improve sensitivity and reliability.

- Even oxygen-free nitrogen contains some oxygen so do not skimp on the oxygen stripping unit.
- All joints and tubing must be gas tight. If possible obtain a couloximeter cell with metal joints rather than glass joints to improve joint seals.
- The injection port septa will usually have to be changed every day and only side vent needles (to prevent septa coring) should be used for injections. Two septa in place of one improves gas tightness.
- Care must be taken to prevent liquid from the desorber passing over into the fuel cell as this reduces efficiency.
- Use the 3-way valve at the top of the couloximeter to seal it off each evening and ensure no oxygen gets to the cell.
- Always run the instrument for an hour before starting runs. If not used for some weeks run the couloximeter for a full day to bring down the baseline (ideally ~2-3µV) before running standards and starting sample analysis.

Further information:

Peck, L.S., and Uglow R.F. (1990). Two methods for the assessment of oxygen content of small volumes of seawater. *Journal of Experimental Marine Biology and Ecology*, 141: 53-62. (Winkler & Couloximeter)

Peck, L. S. & Whitehouse, M. J. (1992). An improved desorber design for use in couloximetry. *Journal of Experimental Marine Biology and Ecology* 163: 163–167.

Microelectrode approaches

The development of microelectrodes has revolutionized the study of micro-gradients in sediments and microbial mats. Of these electrodes, those for oxygen measurements have proved most popular. Three forms of oxygen micro-electrode are available – (a) those based on polarographic measurements

and (b) those based on optical fibre echnology.

a) The original oxygen micro-electrode (or needle electrode) is a simple form of polarographic sensor. Detection is based on diffusion of oxygen through a silicone membrane to an oxygen reducing cathode which is polarized against an internal Ag/AgCl anode. The flow of electrons from the anode to the oxygen reducing cathode linearly reflects the oxygen partial pressure around the sensor tip and is in the pA range. The current can be measured by a high quality picoammeter. Whilst it has a very small tip (a few microns in diameter) the needle electrode is relatively difficult to make and is susceptible to electromagnetic fields, to chemical contamination and to stirring effects. These electrodes have largely passed out of regular use but a robust version mounted in a syringe needle is available from Diamond General Corporation (www.diamondgeneral.com) and has been used in Antarctic studies.

b) The second generation oxygen micro- and minisensors are also polarographic (Clark-type) sensors. Sensing tips as small as 1µm are possible with this design though they are then very fragile. More typically the sensing tip is ~25µm and supported within a syringe needle. Oxygen consumption by the electrode is very low and with an internal guard cathode fitted there is considerable stability. An extra glass housing significantly reduces electromagnetic interference and there is relatively good recovery from sulphide poisoning. Response time is as little as 1-3s, sensitivity is around 0.1µM oxygen and stirring sensitivity is <5% and usually <0.5%. The sensors have a life of around 1 year but personal experience suggests 2-3 years if they are not heavily used. Do not bother trying to make these sensors – it is very difficult. Instead obtain them from Unisense (www.unisense.com.) or Diamond General (www.diamondgeneral.com.). Unisense supply their sensors in various formats, whilst Diamond General offer a relatively bulky glass electrode body which is however robust and very stable.

c)

d) The fibre optic probes or optodes have an oxygen sensitive luminophore incorporated in a hydrophobic polymer which coats the tip of the optical fibre. Light from an LED passes through the fibre and activates the luminophore and oxygen causes quenching of this luminescence. A phase-modulated fluorometer then detects this quenching. Optodes are insensitive to most environmental issues, such as water flow velocity and magnetic fields as well as virtually all chemical inhibitors. Photobleaching can be a problem in smaller tip sizes and optically isolated sensors are available where the environment shows intrinsic fluorescence between 600-660nm (i.e. some mats). The sensor is stable for >1 year, has high sensitivity, consumes no oxygen and has a response time of <1sec for tapered probes. The response is hyperbolic and can be tailored to specific oxygen ranges by using different luminophores. Larger flat ended probes are more photostable and robust but have much slower response times (up to 40 sec) and lower signal intensity. Optode tips are invariably >20µm in diameter. These probes cost substantially less than a polarographic microelectrode but the phase-modulated fluorometer is relatively expensive. Both can be obtained from Presens (www.presens.de).

To insert these micro-electrodes into mats or sediments requires a micropositioner and whilst manual versions (resolution 10-50 µm) can be used in the field (Marzhauser MM3), motorized versions (some battery powered) are more precise (resolution down to 1µm) and easier to use, especially as some can be interfaced to computer control (Davey & Ellis-Evans 1996, Journal of Bryology. 19:235-242). With such capability it is possible to resolve distances twice the tip diameter but more typically ~50µm intervals are employed.

15.2.1.2 Respiration rates and photosynthesis in mats and sediments

Couloximetric measurement of respiration in sediment cores/microbial mats

Place the core or respiration chamber in a water bath or hold in a constant temperature room to maintain the experimental temperature regime. For respiration measurements, wrap the chamber or core with metal foil to prevent ingress of light. It is recommended that at least 250ml of water overlie the core or mat and that <2% of the total water volume is removed for analysis over the incubation period. Depending on the activity of the sediment or mat and the environmental temperature, incubations may last 4-24 hrs. It is important that the oxygen concentration in the water does not fall below ~2 mg l^{-1} over the course of the experiment as the oxygen supply to the sediments or mats can then become limiting. Particularly with sediment cores, insert a manual stirrer in the overlying water through the sampling cap. Before removing each water sample, very gently turn the stirrer to break up any oxygen gradients in the water body, without disturbing surface sediments, and ensure a representative water sample is obtained. In either the sediment core or incubation chamber an injection port is fitted and water samples are obtained via this port using a gas-tight glass syringe and a side-vent needle (to prevent coring of the septum). Care is taken to not draw gas bubbles into the syringe during sampling and, if the couloximeter is not close to hand, the needle tip is pushed into a rubber bung to prevent atmospheric exchange during transit. 500µl aliquots are removed each time and 100-150µl sub-aliquots injected, at least in duplicate, into the couloximeter. Ideally use a reporting integrator and record peak area. The peak is normally a clean spike with a short tail and a timed method can be run in the integrator to semi-automate the recording process.

Photosynthesis measurement in microbial mats using microelectrodes

Early oxygen production based studies usually used modifications of the micro-Winkler technique though couloximetry now offers a more convenient modern method for examining bulk primary production. However to measure photosynthesis within a mat profile, microelectrodes now offer a very rapid light-dark shift method. This is based on the observation that if light is available for photosynthesis and then switched off, the oxygen concentration immediately decreases and the rate of oxygen declines over the first 2-3 s is equivalent to the rate of oxygen production when the light was on. Recording this decline requires that a number of very rapid measurements be taken to allow calculation of the slope by linear regression.

This work is usually undertaken in a laboratory under artificial light, but one can also make such measurements in natural light on cloudless days with solar radiation being logged continuously throughout the experiment. The mat may be overlying a sediment core or have been removed and placed on agar in a petri dish base. Placing the mat on agar eases handling and if the delicate electrode tip is pushed to far through the mat it only moves into soft agar. In the case of a sediment core, the core is pushed up the secured core tube to within ~0.5 cm of the top of the tube and overlying water left in place. In the case of microbial mat placed on agar, water from the sample environment is poured gently over the microbial mat to fill the petri dish base. This prevents dessication and allows for more natural diffusion of dissolved oxygen within the sample material. An oxygen microelectrode is mounted on a thin extension arm in a micropositioner and moved down into the mat to the required depth at an angle of approximately 45° to the vertical. Care should be taken to ensure the area being measured is not shaded by the apparatus. Locating the electrode tip at the surface of the mat is done visually using a telescope or a dissecting microscope head mounted on a support arm.

If the oxygen sensor is an optode it will need to be connected to a phase-modulated fluorometer. If the sensor is polarographic it should ideally be connected to an oxygen meter dedicated for these microelectrodes but can also be connected to a picoammeter. If the latter this will need to be either modified internally or have a circuit inserted between the meter and the sensor to apply a polarization voltage of 0.75V in order to obtain a sensible output. The meter should then be linked to a recording system. Ideally, this should be a datalogger that accepts millivolt output and is set for fast data capture (sub 1 sec rates) in order to obtain at least five readings in 3 secs. These readings can then be put into a linear regression calculation to obtain the slope and therefore the rate of oxygen concentration change. [If a datalogger is not available it is possible to record output on a chart recorder or reporting integrator and either establish the slope of the line by manual calculation or by scanning the plot into a digital file and using software, such as Un-plot, to obtain data points from the original plot.]

To start a set of measurements, place the microelectrode at the required point in the mat and allow the mat to be exposed to sunlight or artificial light and monitor oxygen concentrations, which should rise steadily, on the meter readout, eventually plateauing. Ensure the datalogger is running and then either switch off the artificial light or place a darkroom cloth over the apparatus to block off sunlight. The oxygen curve will immediately drop. Allow this to continue for 10 secs and then remove the cloth or switch the lights on. The oxygen curve will quickly flatten out and then rise back to its original plateau once more. Once it reaches its original high point repeat the process at least twice. The probe can then be relocated at a new position and the procedure repeated as necessary. Calibration of the probe can be made with 0% (using oxygen-free nitrogen or argon to strip oxygen from water) and 100% (bubble air through water) standards at the same temperature as the couloximeter.

Note: - Using a wedge filter moved by a micropositioner across a slot in a guideplate it is also possible to select particular wavelengths with which to expose specific sites on the mat and then measure photosynthesis rates at these wavelengths by light-dark shift. Although somewhat time-consuming this can generate action spectra for different depths in a microbial mat, providing insights to pigment distribution and light capture strategies in these mats.

15.2.1.3 Whole Lake Estimates of Respiration

Use of dissolved oxygen profiles to calculate whole lake respiration in ice-covered lakes was first reported by Welch (1974) and the approach somewhat refined by Barica & Mathias (1979) to cover a wider range of lakes. The approach requires knowledge of the lake bathymetry and ice thickness and requires regular oxygen profiles throughout the period of ice cover. The evidence is that oxygen consumption is essentially benthic and so areal data is more relevant than volume. Barica and Mathias suggest that the reliability of the estimate is significantly influenced by bathymetry and summer lake productivity. It is not a precise technique but gives an acceptable estimate for a range of lake types.

Oxygen profiles

- Establish at least one sampling point. This should certainly be at the deepest point. If possible set up a second shallower site to check for effective horizontal mixing. During early winter when ice is thin this may require setting up two sets of markers at the lake edge to triangulate each sample site location(s).
- On each sampling trip first note the ice thickness and if possible re-establish the water depth in case there has been a change in lake volume.
- If using a polarographic oxygen electrode there is the possibility of the electrolyte freezing at very low air temperatures. Adding ethylene glycol to 25-30% v/v will prevent freezing and appears to have little effect on the electrode performance.
- Take an oxygen profile using at least 1m intervals from the ice surface and ideally 0.2 – 0.5m intervals close to the ice, close to the sediments and across any sharp chemocline. Make sure the oxygen electrode has stabilized at each depth. In

very cold conditions this may take up to 10 min for the first reading so place the electrode in the water to equilibrate whilst checking ice and water depth.

- On return to the laboratory these data can be converted into depth stratum volume values, remembering to correct the top metre stratum for the effect of ice formation. Experiments have shown that essentially 100% exclusion of dissolved oxygen occurs during ice formation. A whole lake volume can then be calculated and converted to an areal measure using the under-ice surface area of the lake. Note - this does equate to summer open water respiration rates.
- There is essentially linear reduction in oxygen content over the early winter and the slope, calculated by linear regression approximates the whole lake winter respiration rate as $gO_2\ m^{-2}\ d^{-1}$ in single basin oligotrophic lakes without an anoxic sump. In more eutrophic systems or those with multiple basins it is recommended to apply a power function transformation ($X' = X^{0.65}$)

Several models for estimating whole lake respiration without oxygen profiling have been developed but no universal model has yet been devised. The equation of Babin & Prepas (Can. J. Fish. Aquat. Sci. 42:239-249, 1985) which incorporates total phosphorus as a measure of productivity appears the most effective for a broad range of different lake types though it significantly underestimates winter respiration in very shallow lake/pools (<2 m mean depth).

Winter oxygen respiration rate ($gO_2\ m^{-2}\ d^{-1}$) = $-0.101 + 0.00247TP_{su} + 0.0134ż$.

In this equation TP_{su} ($mg\ m^{-2}$) is the product of summer total phosphate values in the euphotic zone and the depth of the euphotic zone whilst ż is mean depth (m).

References

Babin, J. and E.E. Prepas. (1985). Modelling winter oxygen depletion rates in ice-covered temperate zone lakes in Canada. *Can. J. Fish. Aquat. Sci.* 42: 239-249.

Barica, J., and J.A. Mathias. (1979). Oxygen depletion and winterkill risk in small prairie lakes under extended ice cover. *Journal of the Fishery Research Board of Canada* 36: 980-986.

Welch HE (1974). Metabolic rate of arctic lakes. *Limnol. Oceanogr.* 19: 65-73

15.2.1.4 Dissolved inorganic C uptake measurements

For measuring the C uptake rates C isotopes are used. A known concentration of the isotope is given to the organisms and the incorporation rate of the isotopes will represent a portion of the total C incorporation. The isotope concentration added will be always at tracer level (10% of the total inorganic C concentration) and will not represent any interference with the natural activity. Two kinds of isotopes can be used, the radioactive ^{14}C, or the stable non-radioactive ^{13}C. The utilization is very similar although the stable isotope (^{13}C) require mass spectrometry and usually larger incubation periods, since the method is less sensitive. The radioactive method is more sensitive but in field or deep field experiments it may be inappropriate or not recommendable.

The methodology of Wetzel & Likens 1991 (Limnological Analyses, Springer) has been used successfully in Antarctic lakes in a modified form. It essentially measures rates closer to net carbon production whereas oxygen production measures gross carbon production but is orders of magnitude less sensitive for phytoplankton studies. 70 ml polystyrene cell culture flasks are usually employed in maritime Antarctic lakes as they have good light characteristics and are relatively robust but Whirlpak bags have also been used by some researchers in continental Antarctic lakes.

- Two experimental bottles and one dark (black tape) bottle (surface, mid-depth and bottom water) or formaldehyde-fixed (2% final concentration) blank (all other depths) were filled with water from each lake depth taking care not to expose the plankton in the water samples to high incident light levels.
- At each depth fill an additional amber bottle with water to overflowing and place

in an insulated box for return to the laboratory and immediate measurements of pH and alkalinity (total CO_2)

- To each incubation bottle add an aliquot of ^{14}C-bicarbonate (4-8µCi depending on the season, specific activity 55 mCi mmol^{-1}, Amersham). An aliquot is retained for later determination of the exact activity of the stock solution. For ^{13}C utilization add 10-50% of DIC concentration as ^{13}C-bicarbonate (98-99% atom ^{13}C)
- Incubations are carried out in situ at the respective sample depths for 2-3 hrs within the period 0900-1500h.
- At the end of incubation, the bottles are recovered and returned immediately to the laboratory in a light tight box.
- For ^{14}C analysis samples are filtered immediately through 0.2µm cellulose nitrate filters under low vacuum (<50mm Hg) and the bottles and filter apparatus rinsed with 5ml of filtered sterile lake water. For ^{13}C analysis samples are filtered immediately through GF/F filters (precombusted at 450ºC for 1.5h)
- The filters are placed in scintillation vials and air-dried for 6h at 20ºC.
- The dry filters are then exposed to fuming hydrochloric acid in a fume hood for 20 min to remove any residual inorganic ^{14}C.
- Total CO_2 content of the lake water samples is measured using the Gran titration method outlined in the Water Analyses section.
- For ^{14}C analysis Filter-Count (Packard) scintillation fluid is added to dissolve the filters overnight and each vial is then counted in a scintillation counter using external standards quench correction to obtain dpm values. For ^{13}C analysis introduce the filter (or a part of it) in the metal capsule and combust in the elemental analyser connected to the mass spectrometer.
- A known small volume of the stock solution is added to scintillation fluid to obtain the original amount of ^{14}C present.
- For calculations one requires (a) the amount of ^{12}C available in mg l^{-1} (obtain from Gran titration), (b) the amount of ^{14}C assimilated in dpm (total filter counts corrected for dark fixation and for the isotopic effect (x1.06)) and (c) the amount of ^{14}C available in dpm corrected for counter efficiency.
- The calculation is then:

- Rate of ^{12}C assimilation = (a) x (b) *1000/ (c) and corrected for incubation time

- For calculations with ^{13}C is required 1) the amount of of ^{12}C available in mg l^{-1} (obtain from Gran titration), 2) the proportion of ^{13}C/^{12}C in the sample (mass spec output), 3) the amount of ^{13}C added for the incubation, and 4) the natural abundance of the sample. An analysis of particulate organic carbon is also recommendable.
- The calculation is:

  Vc (h^{-1}) = %^{13}C-Natural abundance/(((((^{13}C added/(^{13}C added+DIC))*100)-natural abundance)* time)

  Rho C (µg C l^{-1}h^{-1})= Vc*POC (µg C l^{-1})

- Correcting the rate to a daily rate can be done by periodically determining productivity in a series of 4h measurements from dawn to dusk, plotting the resulting curve and integrating the whole curve for comparison to the time period used for regular measurements. The resulting relationship can then be used to calculate daily rates from a single 4hr experiment.
- Vollenweider has proposed from mathematical modelling that the 4h circum midday period represents ~ 30% of the full light day's productivity and that the overall error of such calculations is ± 10% or less.

Extracellular release of DOC can be measured by removing a known volume of filtrate, acidifying it to pH 3 with 3% orthophosphoric acid to remove $^{14}CO_2$ and then freeze-drying if necessary to concentrate the sample before resuspending in 1-5ml of water and adding scintillation fluid for scintillation counting. Generally maritime Antarctic lakes gave extracellular DOC production values of <10% of total production.

C uptake rates can also be measured in microbial mats using similar methodology. In this case we use small cores of the microbial mat, 5-8 mm diameter is a reasonable size to allow a proper penetration of the C isotopes within the mat, although some authors

recommend smaller cores of 1-2 mm diameter. Several cores may be introduced in small Whirlpack bags with 10 ml of pond water and the chosen isotope. The Whirlpak bags are tightly closed keeping a flat shape. The bags are incubated flat. Make sure that the surface layer is facing up and avoid overlapping of cores. An incubation of 2-3 hours is recommended.

After the incubation the Whirlpak bags are kept in light tight container. The cores are collected from the bags with forceps and placed in scintillation vials into which 2 ml of 1N HCl is poured, and are kept overnight. Then 2 ml of 1N NaOH is added to neutralize the acid, and if ^{14}C was used the vial content is sonicated until total disruption of the mat is achieved. An aliquot of the paste is obtained with a wide-mouth tip pippette and scintillation liquid is added. It is necessary to build quenching curves with the same mats not exposed to radioactivity and adding known radioactivity amounts. If ^{13}C is used, then the cores are collected from the liquid with forceps dried in paper and frozen until analysis is made. The core is treated as above for C and N analysis.

Reference
Wetzel RG & Likens GE (1991). *Limnological Analyses*. 2nd. Ed. Springer-Verlag. 391p.

15.2.1.5 Anoxygenic photosynthesis

(Antonio Camacho)

Under certain circumstances anoxygenic photosynthesis can be relevant, especially when studying microbial mats and anaerobic habitats. The anoxygenic photosynthesis is estimated adding DCMU, which blocks the oxygenic photosynthesis. Under these conditions the C assimilation is mainly due to anoxygenic photosynthesis.
The methodology is very similar to previous ones. Although both isotopes may be used , ^{14}C is recommended, because of its higher sensitivity. One hour before the isotope is added, DCMU (Dupont) is added to obtain a final concentration of $10^{-5}M$. Subsequently follow the same procedure as above.

15.3 BACTERIAL PRODUCTION

(*Cynan Ellis-Evans*)

These are usually involving thymidine or leucine incorporation or both. Adenine was also proposed originally but has fallen out of favour. The thymidine approach quantifies DNA synthesis rates in heterogeneous populations of aquatic bacteria whilst leucine quantifies protein synthesis. Both methods are based on generalized assumptions and therefore have some limitations.

15.3.1 [Methyl-^{3}H] Thymidine Incorporation

- Add 10ml of water sample to a 20 ml glass scintillation vial.
- Add ^{3}H-labeled thymidine to give a final concentration of 15-20 nM.
- Stopper each bottle firmly.
- If incubating in the field, put the bottles inside a plexiglass tube, seal both ends and lower to required depth.
- Incubate for 30 min at environmental temperature.
- Stop the incubation by addition of formaldehyde to a final concentration of 1-2%.
- Allow to stand for at least 15 min and store in a refrigerator for no more than 24 hrs before processing.

There are two methods of extraction. The incorporation into total macromolecules method is commonly used and is relatively convenient to do in the field as it uses only trichloroacetic acid (TCA) and ethanol washes. It is not however as reliable as the incorporation into DNA method, which employs phenol-chloroform which can be hazardous to use in the field.

As this technique involves a lot of samples and filtration rates are slow, try to have a multiple filtration manifold unit with stainless steel funnels taking 25mm or 47mm diameter 0.2µm cellulose nitrate filters (not cellulose acetate as these dissolve in phenol:chloroform) to permit simultaneous filtration of up to 12 samples. If a manifold is not available a standard glass 25mm or 47mm diameter filter unit can be used. As the DNA incorporation method uses phenol-chloroform do not use commercially available plastic filter units in this method.

Incorporation into total macromolecules

- Keep samples chilled at all time with an ice bath or a refrigerator.
- Place the required number of filters in distilled water containing ~1mM non-radioactive thymidine in a petridish. Presoaking gives lower and more uniform blanks.
- Put the filter units in a fridge to chill down.
- Add 50%TCA solution to give a final concentration of ~5%, stopper and shake well.
- Keep on ice for 20 min and then shake again.
- Assemble the filtration equipment and insert presoaked filters.
- Carefully pour sample into each funnel and filter at < 100mm Hg pressure.
- Rinse each vial with 2ml of ice-cold 5% TCA and add the rinse to the relevant funnel.
- Rinse each funnel 3 times with 1ml aliquots of ice-cold 5% TCA dispensed through a fine pipette to ensure the funnel sides are well washed.
- Rinse the filters with five 1ml aliquots of ice-cold 80% ethanol to remove thymidine incorporated in cells but not incorporated into DNA.
- Remove the vacuum and carefully lift each filter off the filter unit and place in a scintillation vial. The filter can then be stored frozen for several months.
- To dissolve the filter one can use Filter-Count (Packard) scintillation fluid.
- Otherwise add 1 ml of ethyl acetate to dissolve the filter and after 30 min add 10ml of your favourite scintillation fluid.
- Count in a scintillation counter, making an appropriate quench curve to allow conversion of cpm to dpm.

Incorporation into DNA

- Add 5M NaOH to the water sample to give a final concentration of ~0.25M. (this step can replace the formaldehyde fixation step, particularly for lab incubations)
- Keep at room temperature for 1 hour whilst this step removes RNA.
- Cool the samples and then acidify to pH1 with 1ml of chilled 100% TCA. Shake well and keep chilled in an ice bath for 15 min.
- Place presoaked filters into chilled filter units and filter samples at <100mm Hg.
- Rinse each vial with 1ml of ice-cold 5% TCA and add the rinse to the funnel.
- Rinse each funnel carefully three times with 1ml of ice-cold 5% TCA.
- Rinse with 5ml of chloroform-phenol solution (50% w/v solution, 50g of phenol in 100ml of chloroform) to remove protein.
- Rinse filter with five 1ml aliquots of ice-cold 80% ethanol.
- Remove the filter carefully and place in a scintillation vial. The filters can be stored frozen for several months prior to counting.
- Either dissolve with ethyl acetate and then add scintillation fluid as above or simply add Filter-Count (Packard) scintillation fluid which dissolves the filter.
- Count in a scintillation counter with an appropriate quench curve to give dpm.

^{3}H-thymidine solutions can be obtained from Amersham (code TRK418) with a specific activity of 40-80 Ci mmol^{-1} and this must be stored at 2°C and ideally used within 2 months. Otherwise add 10% ethanol to reduce the self-decomposition rate. However if adding 10% ethanol, it will be necessary to evaporate the solution to dryness using sterile filter air and reconstitute in sterilized water.
The working solution should be made using sterile distilled water so that each 100µl addition to 10ml water samples contains 20 nM of thymidine. If storing the working solution for more than one week add ethanol to 2% final concentration. Do not autoclave the working solutions and instead filter through a sterile 0.2µm syringe filter unit.
Do not allow thymidine solutions to freeze and note that filter apparatus and solutions should be chilled but not freezing. For field work transport the thymidine solutions in a thermos. To remove possible errors associated with label being trapped near the edge of the filtering area, Richard Robarts recommends a circular cutter to remove the non-filtering part of each filter plus 4% of the filtering area. The scintillation counter can be programmed to increase the counts by 4% for the portion of filtering surface excised. This procedure gives less variability between replicate filters.

Bacterial productivity calculations based on thymidine incorporation

Rates of thymidine incorporation are converted into moles of thymidine per unit volume to permit comparison. A conversion factor (2 (or 2.5) x 10^{18} cells per mole thymidine) is commonly used to convert moles incorporated to cells produced in unit time. To then convert cells produced into carbon production requires knowledge of the total number of bacteria in each sample, the mean cell volume (MCV) of these bacteria and a further conversion factor of 350fg C µm^{-3} of cell volume (Bratbak 1993).

Moles incorporated l^{-1} h^{-1} = ((sample dpm-blank dpm) x (4.5 x 10^{-13})/SA x T x V) x 1000.

Where 4.5 x 10^{-13} is the number of curies per dpm; SA is the specific activity of thymidine in curies mmol^{-1}; T is incubation time (h); V is the filter volume in litres.

The moles incorporated in unit time are converted to carbon production using:

Production (µg C l^{-1} h^{-1}) = (moles incorporated) x (2 x 10^{18}) x (carbon per average cell volume).

The total bacterial count and MCV can be obtained by DAPI or Acridine Orange epifluorescence, but it is acknowledged that the total count includes cells not incorporating thymidine. Using either microautoradiography of ^{3}H-thymidine uptake or the total viable count obtained with CTC epifluorescence (Del

Giorgio & Scarborough, J Plankton Res. 17: 1905-1924, 1995) may give a more realistic assessment of the bacteria actually involved in C production.

15.3.2 Leucine Incorporation into Protein

Leucine comprises a relatively constant fraction of bacterial protein. By knowing the ratio of protein to total biomass it is possible to calculate biomass production without the need for cell volume measurements used in the thymidine incorporation method. However both methods have limitations and results obtained must be interpreted with care.

Procedure

- All glassware must be acid-washed to ensure no contamination from amino-acids.
- Place 20ml of water sample in each of four 30ml glass Universal bottles
- To each bottle add ^{3}H-Leucine to a final concentration of 10nM.
- To the fourth bottle immediately add 50% TCA solution to give 5% final concentration. This bottle will be the fixed control.
- Incubate for 2 - 5 hours at in situ temperatures (in field or lab).
- Add 50% TCA to the three experimental bottles to obtain 5% final concentration. This stops the incubation and starts the extraction. The original method suggested heating the sample to 80ºC for 15 mins but in practice this heating step is usually unnecessary.
- Filter the sample through a 0.45 µm cellulose nitrate filter at < 100mm Hg pressure.
- Rinse each filter twice with 3ml of cold 5% TCA.
- Rinse each filter twice with 2ml of cold 80% ethanol.
- Remove the filter towers and gently rinse the filters with 1ml of 80% cold ethanol.
- Place filters in glass scintillation vials and add Filter-Count (Packard) scintillation fluid to dissolve the filters before counting in a scintillation counter against an appropriate quench curve for dpm.

Obtain [4,5-^{3}H]-leucine with a specific activity of 40-60 Ci mmol^{-1} from New England Nuclear or Amersham International.
The equation relating leucine incorporation to biomass production is:

$$\text{Production (g C l}^{-1}\text{ h}^{-1}\text{)} = \text{Incorporation Rate (mol l}^{-1}\text{ h}^{-1}\text{)} \times 3.1$$

where 3.1 kg C mol^{-1} is a conversion factor based on a molecular weight of 131.2, that the fraction of leucine in protein is 0.073, the ratio of cell carbon to protein is 0.86 and the isotope dilution factor is 2.

15.3.3 Dual Labelling of Thymidine and Leucine

The thymidine incorporation method is subject to a number of potential errors, most notably the possibility that observed rate changes are in fact variations in the conversion factor over time. One way to establish what is a real change is to run a second independent production assessment (in this case leucine incorporation) in parallel. The two methods should co-vary over time and space but can differ significantly at times of unbalanced growth when the rates of macromolecular synthesis are uncoupled. Both methods are dependent on conversion factors that potentially introduce further errors but nevertheless these procedures are the most effective currently available.

Procedure

- For each water depth there are triplicate 30 ml polystyrene Universal sample bottles and a fixed control (50% TCA added to a final concentration of 5%).
- To each bottle add 10-20nM (final concentration) of [^{3}H-methyl]-thymidine (specific activity 40-60 Ci.mmol^{-1}, Amersham International) and 10nM (final concentration) of L-[U-^{14}C]-leucine (specific activity ~300mCi.mmol^{-1}).
- Incubate for 1 hour either in situ or at environmental temperatures in the laboratory.
- Stop further activity by addition of

50% ice-cold TCA to a final concentration of 5%.

- After allowing the bottles to stand in the refrigerator for 20 min to allow full extraction, the contents were filtered through 0.2um cellulose nitrate filters at <100mm Hg pressure.
- The sides of each filter unit were rinsed with 1 ml of ice-cold 5% TCA and the filters washed with a further 1 ml of 5% TCA.
- The filter funnels were removed and the filters washed with three 1 ml aliquots of distilled water.
- The filters were then transferred to glass scintillation vials and dissolved using Filter-Count scintillation fluid before counting in a scintillation counter in dual label mode.

A conversion factor of 2.5 x 10^{18} cells per mol^{-1} is applied to thymidine data, and mean cell volumes are calculated from DAPI epifluorescence preparations. The conversion factor used for bacterial biomass to carbon content is 350fg C.μm^{-3} though a value of 220fg C.μm^{-3} has been used by some workers as a more conservative estimate.

The concentration of ^{3}H-thymidine added is dependent on the environment. In each case a saturation curve experiment should be undertaken to establish the amount needed for maximum uptake. In both an ultra-oligotrophic continental Antarctic freshwater lake and in oligotrophic and eutrophic maritime Antarctic lakes 20nM final concentrations were needed. This is not an issue for leucine.

Reference

Chin-Leo, G., D.L. Kirchman. (1988). Estimating bacterial production in marine waters from the simultaneous incorporation of thymidine and leucine. *Appl. Env. Microb.* 54:1934-1939.

15.4 SEDIMENT TRAPPING AND CARBON/NITROGEN ANALYSIS

(*Cynan Ellis-Evans*)

There has been considerable research on sediment trapping in different environments and our choice of trap design reflects this research. Sediment trapping provides valuable data on the seasonality, quantity and composition of sedimenting material. These traps are normally placed at the deepest point in a lake but additional traps placed near the inflow will give information on allochtonous inputs during spring melt.

- The most efficient trapping design is a simple cylinder, open at the top. All other trap designs are less efficient – either under-trapping or over-trapping.
- The cylinder should not be < 4cm as narrow tubes under-trap inorganic material.
- Where there is no clear information on settling fluxes and current velocities (i.e. most lakes) then a simple cylinder with an aspect ratio (height : internal diameter) of 3:1 or 4:1 is most appropriate.
- At greater velocities the aspect ratio has to increase to ~ 5:1 to prevent resuspension of trapped material.
- For the simple cylinder design to be effective the tube must be vertical in the water column and if necessary weight applied at its base to ensure this occurs.
- Triplicate or quadruplicate traps are used at each depth. These can be placed in a Perspex plate at each depth for ease of handling.
- Where sedimentation rates are low (under ice over winter) the contents of replicate traps may need to be combined.
- In maritime Antarctic lakes, copepods may on occasion enter the traps and feed on the seston material. Putting a fixative such as formaldehyde at the base of a trap stops any bacterial action and kills copepods, which can then be removed by hand before analysis. The addition of chemical preservatives can compromise certain analyses so it is better to leave traps untreated and not deploy them for more than 10-15 days.
- Whilst the aspect ratio recommended limits resuspension care should be taken to raise traps slowly and smoothly and most importantly not leave them bobbing at the surface of the lake but remove them to a vertical position (in a transit box) and cap them immediately.
- If traps are placed in anoxic conditions, care must be taken to avoid air being trapped in the cylinder during capping. Subsequent treatment should be undertaken ideally in an anaerobic cabinet or in an anaerobic sample handling glove bag. Otherwise precipitation of ferric oxyhydroxides following oxidation may result in errors.
- Where anoxia is not an issue, it is possible to carefully decant the bulk of water from each trap and remove the sediment material with minimal wash water to preweighed evaporating dishes or conical flasks. Samples can be air-dried and reweighed before further analysis.
- Our approach was somewhat different as we had both oxic and anoxic samples and wanted to use a CHN analyzer. The total content of each trap was therefore filtered through pre-dried (60ºC) and pre-weighed 0.45µm Nucleopore filters. Filters were then dried and reweighed before storing. Where sediment quantities were low, replicate traps were combined on a single filter.
- A cork borer was used to remove 5-10mm diameter discs of material from the filters and these (filter and particulates) were weighed and placed in tin capsules for analysis in a Carlo-Erba CHN analyzer. Tests using 20 discs from 20 different filters in the same production batch have shown negligible intra- or inter-filter variation in CHN content so the CHN values of blank filters could be subtracted from sample filters.
- Sulphanilamide was used as the analyzer standard
- Summing the CHN content of each filter always resulted in substantially less than 100% of dry weight. This difference (residual) represented the bulk of dried material. Where sufficient sedimentation occurred parallel ashing experiments (475ºC for 24h) revealed a strong linear correlation with the residual values (r^2 = 0.94) so the residual was the inorganic component.

16 N-cycle

(Antonio Quesada)

The protocols for the N cycle are based on studies of microbial mats, where large biomass is available and habitat heterogeneity allows most of the biological processes related with this cycle.

16.1 N_2-FIXATION

Several techniques are available but the most appropriate for polar environments is the acetylene reduction one. This technique is based in the capability of the nitrogenase enzyme to reduce acetylene to ethylene. The sample is exposed to acetylene and the production of ethylene is proportional to the nitrogenase activity and thus to N_2-fixation. As this determination is made with gases (acetylene and ethylene) all the material used has to be gastight.

For the incubation we have found the flat tissue culture flasks very convenient because of their optical characteristics. These flasks (in any of their sizes) can be stoppered with reversible latex gastight stoppers, providing a good incubation chamber. Place 3 to 5 mat cores (15-20 cm diameter) in a 275 ml flat flask with 100 ml of GF/F filtered pond water, and place the gastight stopper. Extract with a syringe 10 % of the air volume (27 ml) and inject the same volume of pure acetylene. The acetylene can be purchased pure in metal cylinders (heavy and complicated to transport) or can be prepared from calcium carbide. In any case, the acetylene can be transported to field sites in gastight bags provided with a septum. The diffusion constant of both acetylene and ethylene in water is quite low. This necessitates vigorous shaking (without breaking the cores) after adding the acetylene and before getting the gas aliquots. Take an aliquot of the gas added (time 0) by using 10 ml Vacutainer vacuum tubes (Becton Dickinson) and double needle (Becton Dickinson). First place the needle in the flask and then press the rubber stopper of the vacuum tube against the other side of the needle. Leave in this position for at least 1 minute. The vacuum tubes must be 'dry' (with no additives, since these additives can react with gases). The tubes must always be the same brand and type since the vacuum differens between brands and types. In any case, with every batch of tubes a determination of the vacuum inside is needed. This can be estimated by high precision barometers or by dilution, injecting a known concentration of any gas and measuring the dilution of this concentration by gas chromatography. Typically the dilution observed in the available brands ranges from 10 to 20 %. Once the aliquot has been taken, place the flask flat and submerged at the pond shore, making sure that the surface layer of the mat is facing up in the bottle, and avoiding overlapping of the cores. Remember that most likely N fixation in this environment is photodependent. After 4 hours of incubation shake vigorously and take two gas aliquots with double needle and Vacutainer vacuum tubes. Note that longer incubation periods are not representative of the nitrogenase activity since cells are poisoned with acetylene after 7 hours of incubation, and from that moment no further accumulation of ethylene takes place.

The gas samples can be kept at ambient temperature for at least one month without changes in the concentrations. Once in the laboratory, the gas is analysed by gas chromatography with flame detector and a Porapak Q, using standards for ethylene. One can estimate the ethylene produced by the organisms from acetylene. The calculations are made using the surface of the cores in the flask, the total volume of the flask (including the water phase), the dilution by the vacuum tubes, the volume injected and the incubation time. The time 0 sample is important to subtract the amount of ethylene introduced with the acetylene, since trace concentrations of this gas are usually present in the acetylene.

The conversion from ethylene produced to N_2 fixed is still under discussion, since molar ratios acetylene/N from 2 to 12 have been published for cyanobacteria. Our results with field populations of cyanobacteria, using $^{15}N_2$ indicate that a ratio of 4 or 5 is quite close to reality.

16.2 UPTAKE OF INORGANIC N COMPOUNDS (AMMONIUM AND NITRATE)

Nitrate and ammonium uptake are also good variables related to the N cycle and the N status of the community. The methodology explained here is related to microbial mats, but can also be used for planktonic communities.

Take 3 cores of 10 mm diameter, and place them in small Whirlpak bags with 10 ml of GF/F filtered pond water, and add the ^{15}N compound (potassium nitrate 99% atom or ammonium sulphate 98% atom) at tracer concentration. Close the bag and incubate at pond temperature for 4-5 h. Then, collect the cores with forceps, clean in filtered pond water and dry in Whatman paper, keep the cores on paper and freeze until analysis. The treatment of the core is identical to C and N determination in cores, except that the isotope abundance is determined by mass spectrometry.

16.3 DENITRIFICATION

Denitrification is one of the ways N is lost from the ecosystem, and consists in the transformation of nitrate into N_2, which is lost as a gas into the atmosphere. This process requires anaerobic conditions and can be quite common in microbial mats, where an anaerobic layer is typically present. Commonly nitrification and denitrification are fully coupled and this is the reason why nitrate concentration is extremely low in many ponds carpeted with microbial mats. Therefore, the denitrification rate may be an important process in the N cycle of the ecosystem.

The technique described here deals with the potential denitrification, using nitrate in excess. Two determination techniques are available, one using acetylene as a blocking reagent of the last step in the denitrification which produces N_2O which is measured by gas chromatography, and the other uses $^{15}N\text{-}NO_3^-$ and measures the $^{15}N\text{-}N_2$ produced in the process.

16.3.1 Acetylene technique

This method requires bottom-open incubation chambers. These chambers should be made of non-toxic transparent plastic, and it should be possible to close them gastight. Five-litre mineral water bottles have been used with excellent results. These are cheap and disposable, and easy to prepare by cutting off the bottom and stoppering with a reversible rubber gas-tight stopper. The advantage of this technique is that it can be done in situ without disturbing the mat structure and thus the oxygen balance.

The incubation chamber is inserted into the mat and sediment at least 5 cm. Note that this method cannot be used in gravel sediments because the chamber will not be gas tight. Close the chamber and inject a solution of potassium nitrate at a concentration of about 2 g $N\text{-}NO_3^-.m^{-2}$, with respect to the surface area of the mat inside the chamber. Immediately extract with a hand pump 500 ml of air from the chamber and introduce the same volume of acetylene (see the nitrogen fixation section above). After 10 min collect a gas sample in Vacutainer vacuum tubes (see above). After 12 and 24 h of incubation again collect gas samples in triplicates and store until analysis is performed. Note that this technique introduces a large amount of nitrate in the system, although it will probably be denitrified fairly rapidly. But avoid using this technique in extremely sensitive systems. Alternatively this technique can be used in completely closed chambers, placing the nitrate and the biological material in disposable bottles. Closed chambers need to maximize the surface/-volume ratio, and minimize the disturbance of the mat community. Thus chambers should be designed in which a large patch of intact mat can be placed and which subsequently can be closed gas-tight. When the biological material is collected the whole system should be considered, including the anaerobic sediment under the photosynthetic layers. Before starting the experiment the anaerobic conditions should be naturally reconstructed. Microelectrode measurements indicated that after 24 h the mat can be considered very similar to its natural conditions, and the experiment can be initiated in the same way as below, leaving the chamber in the pond.

The gas samples are analysed by gas chromatography detecting N_2O produced after the blocking activity of the acetylene. For detecting N_2O an electron capture detector is needed, but these detectors are poisoned by the acetylene used. A valve system is needed to detour the acetylene before reaching the detector to avoid damage. Typically N_2O comes earlier in a Porapak N column (about 1.5 min) and acetylene takes at least 3.5 min, leaving time to change the valve. Electron capture detectors need to be standardised very frequently, and it is advisable to inject one standard every 6-8 samples. The moles of N_2O obtained in the gas sample, in relation to the mat surface included in the chamber, the volume of the chamber, the incubation time, the dilution of the vacuum tubes and the volume injected in the chromatograph will give the potential denitrification rate in the system in mol of N per m^2 per h, assuming that the substrate is not limiting. One of the triplicates of each sample can be used to assess the gas tightness of the chamber by measuring the acetylene in the chamber by Flame Ionization Detector gas chromatography.

16.3.2 ^{15}N technique

This technique may require the use of completely closed chambers, because in many polar places the release of ^{15}N compounds to the ecosystem is not allowed. Once the biological material has been included in the incubation chamber and the natural oxygen distribution has recovered, close the chamber and inject a solution of $^{15}N\text{-}NO_3^-$ of 99% atom ^{15}N at a concentration of about 2g $N\text{-}NO_3^-$ m^{-2}. After 12 and 24 h collect replicate gas samples in vacuum tubes. Analyze the gas samples by mass spectrometry.

17 S-cycle

(Cynan Ellis-Evans)

17.1 SULPHATE REDUCTION

This can be an important process in polar lakes, particularly where high sulphate levels are present, when they can outcompete methanogens for both acetate and hydrogen. Sulphate reducing bacteria appear to be involved as fermenters in providing acetate to methanogens where sulphate concentrations are low and appear to have a role in nitrogen cycling. Rate measurements are almost invariably made with radio-isotopes though rates can also be calculated in sediments using sediment diffusion coefficients and sulphide profile measurements. Radio-isotope measurements originally focused only on acid-volatile sulphide (AVS) but it is now recognized that non acid-volatile sulphide (NAVS) is also an important component, being rapidly created during sulphate reduction. Modern methods measure AVS and NAVS as a single component as the AVS:NAVS ratio varies with environment (usually 1:1 or 1:2 but the ratio can be larger on occasion).

^{35}S-Sulphate reduction

Labelling and incubation

- Mini-cores (5-6 cm long) are obtained from a large sediment core using 10ml syringes with the luer end removed and sealed underwater with a subaseal . The syringe bodies have previously been marked up at 1cm intervals, holes drilled at these intervals and plugged with silicone sealant.
- 20-50µl of ^{35}S-labelled sodium sulphate (specific activity ~47 TBq (~1.2 Ci) mmol^{-1}), giving a final known concentration of ~ 2µCi, is added at 1-2cm intervals along the core length.
- One core is immediately frozen at -80ºC as a control. Early studies using chemical fixation with concentrated alkali or zinc acetate gave variable results.
- Remaining cores are incubated in a tray of water (to minimize gas diffusion across the syringe wall) at in situ temperatures and in the dark for up to 7 hours.
- During the incubation period the control cores are cut into 1-2 cm sections and analyzed for total sulphate content.
- At the end of incubation all experiment cores are frozen at 80ºC.

Processing of samples

- Frozen cores are cut into 1 or 2cm sections and placed in round-bottomed digestion flasks, previously flushed with OFN and connected via the gas stream to two trapping bottles in series, each containing 40ml of 1% zinc acetate (if simply measuring acid-volatile sulphide).
- 10mls of concentrated HCL is added to each flask and the mixture heated at 40ºC for 30 min. Acid-volatile hydrogen sulphide is swept out of the flask and is trapped as zinc sulphide in the two traps.
- If measuring both AVS and NAVS the above two stages can be omitted. For total sulphide (AVS and NAVS) three zinc acetate traps in series are recommended. The third bottle is found necessary as trace activity is sometimes detected beyond the second trap during processing.
- 10g of granulated tin and 50ml of 20% (w/v) stannous chloride in concentrated HCl are added to each flask and the sediment heated at 100ºC under reflux for one hour to release AVS and more particularly NAVS and flush the sulphide into the trapping bottles.
- Remove 2ml of zinc sulphide solution from each trap and add to 2ml of Packard Instagel scintillation fluid to suspend any particulate material.
- Leave to stand overnight and count in a scintillation counter against an appropriate quench cure to obtain dpm.
- Analyze the remaining sulphate content of each sample flask (using the Tabatabai barium-gelatin method – see water analysis section). Combining it with the trapped ^{35}S-sulphide data gives the total sulphate

concentration in the sample.

- Knowing the initial activity added (A) and the activity of label recovered as sulphide (a) the ratio a/A gives the % incorporation. Knowing the total concentration of sulphate in each sample, the amount of total sulphate reduced in unit time can be calculated.

The methodology is found to yield ~90% efficiency and results are corrected accordingly.

18 Methanogenesis

(Cynan Ellis-Evans)

18.1 METHANE DETECTION BY GAS CHROMATOGRAPHY

Methane is readily quantified by gas chromatography (GC).

- Cheapest method employs a thermal conductivity detector (TCD) with a stainless steel column (6 ft x 1/8 inch) packed with say 80/100 mesh Spherocarb and can detect down to ~10^{-3} atm CH_4 when using helium as the carrier gas.
- The more expensive flame ionization detector (FID) with a 6 foot x 1/8 inch stainless steel column packed with 80/100 mesh Poropak Q and operated at an oven temperature of 50°C and carrier flow rate of 30 ml min^{-1} has a lower detection limit ~10^{-6} atm CH_4. FID is the detection method of choice.

In practice one can use a whole range of packings including Chromasorb or Molecular Sieve, which is cheaper than Poropak. High resolution PLOT capillary columns can also be used but this simply increases the complexity and cost of the gas chromatograph.

A useful addition to any gas chromatograph is a reliable gas sampling valve fitted with 1 or 2 ml sampling loop through which 2-5 ml of gas sample can be easily injected and ensures a consistent sample size. Otherwise use a gas-tight syringe with a side port needle to avoid coring of gas septa and simply inject known volumes into the injector port.

- For sediment incubation experiments at *in situ* temperatures – we use sediment cores in sampling tubes with at least 300 ml of overlying water and a stirring bar fixed in a cage attached to the base of the tube cap. Using another magnet the stirrer can be dragged around slowly to mix the water column before sampling without resuspending sediments.
- 2ml water samples are removed through a side port in the tube at time zero and every 12 hours for up to 3 days with a 5ml glass syringe. For Signy lakes methane production is usually linear for up to 50h. A second syringe fitted to the tube cap can provide a volume of deoxygenated water to replace that removed by each sampling
- For water samples where methane concentrations may be much lower a known volume of water (~30ml) can be drawn off with a large (60 ml) thick-walled plastic catheter syringe or into a Supelco Pressure-Lok glass syringe with zero dead volume. The catheter syringe has the advantage of being cheap and easily connected to an oxygen-free nitrogen gas line. Unlike most plastic syringes these 60ml catheter syringes are gas tight for periods of several hours.
- Add 0.1 ml of 0.5M NaOH to each syringe so raising pH to 11 to prevent methane oxidation.
- A subaseal over the tip provides a gas tight seal on the catheter syringe. On the Pressure-Lock syringe the gas lock is part of the design.
- Draw in a volume of OFN gas equal to that of the water sample in the syringe, reseal and shake for 1 minute before leaving the sample to equilibrate at a standard temperature (20°C). Always use the same gas as is being used as carrier in the GC to avoid blips in the chart record.
- The catheter syringe can then be connected by tubing to the gas sampling valve and at least twice the volume of the sampling loop pumped through to ensure a realistic sample. With a 60 ml syringe (30 ml sample) and 1-2 ml loop between 3 and 5 replicate runs are possible. The Pressure-Lock can be either pressurized and injected directly into the injection port, or flushed through the sampling valve. For sediment core gas samples use a 0.5ml loop. Turn the sampling valve switch to transfer the known volume of gas into chromatograph gas stream.
- The methane peak will come through within a minute of injection in most cases.

- Commercially available gas standards in spray cans can be injected through the sampling loop to calibrate peak heights.
- The proportion of dissolved methane transferred to the gas phase in syringe stripping of a 1:1 gas:water mixture is calculated from Henry's Law to be 96.8% at 20°C. So multiply by 1.032 the methane concentration calculated from the calibration curve to obtain the gas concentration dissolved in the sample. For other temperatures use the formula:

$$X = m / 1+(\alpha V_1 / V_g)$$

where V_1 is the volume of the liquid phase, V_g is the volume of the gas phase, α is the solubility of methane at STP (0.0332ml ml^{-1} water at 20°C) obtainable from tables, m is the volume of CH_4 at STP present in V_1 and X is the volume of CH_4 at STP present in V_g.

18.2 RADIOISOTOPE DETECTION OF METHANOGENESIS IN SEDIMENTS

Relative contributions of acetate and CO_2 to methanogenesis can be assessed using radiotracers. In most freshwaters acetate is the major source of methane but CO_2 is also significant. Other organic sources are also known but are not thought to be important in polar lakes. The natural concentrations of acetate need to be known to a) establish isotope additions are at tracer levels and b) calculate real rates of CH_4 production from acetate. Acetate and other volatile fatty acids can be extracted and analyzed by capillary gas-liquid chromatography (see below). CO_2 is invariably present in high concentrations so additions should always be at tracer level.

Methanogenesis can be measured using cores or slurries. Cores preserve the natural interactions and gradients but for radio-isotope work can be difficult to inoculate effectively. Slurries are easier to work with but the community interactions and natural gradients are disrupted. Both methods have pros and cons but we favour the use of cores.

- Prepare small Perspex tubes approximately 6 cm long to hold a 5 cm sediment sample when seals are inserted at both ends. Drill holes at 1 cm intervals in a spiral along each tube length and seal with silicone sealant.
- Use the small tubes to obtain sub-cores from a larger sediment core and ensure the ends of the small cores are sealed with no headspace whilst under the water surface to maintain hydrogen concentrations.
- Inoculate at 1cm intervals with 20-50µl of $^{14}CH_3COOH$ (~3µCi, final concentration 0.5-1.0 µmol l^{-1}, specific activity 50-60 mCi.$mmol^{-1}$) or $NaH^{14}CO_3$ (~0.8 µCi, final concentration 10 – 20 µmol l^{-1}, specific activity ~50mCi.$mmol^{-1}$).
- Cores are incubated for 24h at *in situ* temperatures in the dark and under water to minimize gas exchange through the minicore wall. Reaction is stopped by placing the cores at -80°C.
- The frozen cores are cut into 1 cm sections, placed in pre-weighed vials and weighed before adding 0.5ml of 10M NaOH to keep $^{14}CO_2$ and $^{14}CH_3COOH$ in solution, sealing with crimped septa caps and allowing the sample to thaw overnight at 20°C.
- The samples are gently shaken for 10 min and then 2ml of headspace (containing labelled methane) removed to a sealed scintillation vial filled with Packard Instagel scintillation fluid – recovery of $^{14}CH_4$ is ~ 70%.
- 2ml of 1.5M sulphuric acid is added to the sample vials and the vials again gently shaken for 10 min. 2ml of the headspace (containing $^{14}CH_4$ and $^{14}CO_2$) is transferred to a sealed scintillation vial containing 0.5ml of ethanolamine and allowed to stand overnight.
- The vials containing ethanolamine are then flushed with argon for 2-3 min to drive off $^{14}CH4$ and Instagel solution added. $^{14}CO_2$ trapping efficiency is ~ 87%
- Both sets of vials are counted in a scintillation counter. Total $^{14}CO_2$ and $^{14}CH_4$ in each vial are calculated using Henry's Gas Partitioning Law and published values of the constant k.

18.3 MEASUREMENT OF SEDIMENT ACETATE CONCENTRATION

- Sediment is sectioned at 1cm intervals and a known weight placed in a centrifuge tube and centrifuged at 6000 rpm for 5 min.
- A known volume of the supernatant is added to 2ml glass autosampler vials and dried out under vacuum.
- The residue is made up to 200µl with 1% HCl.
- 1µl samples are analyzed using an FID-equipped GC with autosampler. A Nukol Megabore (0.53mm i.d.) column coated with cross-linked polyethylene glycol. GC conditions are helium carrier flow of 8ml min^{-1}, isothermal oven temperature of 120ºC, injector at 85ºC and detector at 120ºC.
- Calibration is made using acetate standards containing 0.1, 0.2, 0.5 and 1.0mM of the fatty acid as its sodium salt.

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RiSCC Manual - ANNEXES

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ANNEX 9. **Description of aquatic non-marine systems for non-limnologists** (Basic level)

ANNEX 10. **Fresh and brackish water antarctic algae literature**

ANNEX 11. **Protozoans literature**

ANNEX 1: Observation list (Basic level)

It may be useful to add information on the size of the area studied, as well as some habitat characteristics, e.g.soil depth, moisture and nutrient status, exposure, and saltspray. See the information given with the vegetation description form (annex 3) and Bergstrom & Selkirk (2000) for ordinal scales for these variables.

Observation / Collection Form

Observer / collector: Date:

SCAR Gazetteer place name:

Identifier:

Co-ordinates:

Lat/long? UTM ?

Co-ordinates determined with: GPS Map

If GPS? Reliability score: m

If Map? Scale

Altitude m Estimated/Measured

Depth m Estimated/Measured

Distance from coast: m Estimated/Measured

Slope / Aspect: Human impact?

Site description:

Species name	Presence/ Absence	OR Abundance	OR % Cover

Vascular plants, Mosses, and Lichens:

R: rare; < 5% cover

O: occasional; 5 - 25% cover

C: common; 25 - 75% cover

A: Abundant; 75 - 100% cover

OR % cover only

Invertebrates and Microbial: Abundance/area

ANNEX 2. Site Description for active layer monitoring - additional data

(Basic level)

INSTRUCTIONS

This form is used in addition to the general observation / collection form to provide additional data for sites where active layer monitoring is planned.

Site identifier: use the same identifier as on the general site description form

Samples: indicate if samples have been collected or not, the specific characteristics and sample list can be reported at the end of the form.

Photos: preferably list an identifier for any pictures taken from the site

Geomorphological characteristics: provides a general indication of the geomorphological characteristics of the community location within the site.

Rock type: indicate the type of rock, if available, ands if the rock is silicious or calcareous

Soil Typology: indicates soil absence or presence and, if present, its main nature. Soil absence is meant mainly on rocky habitats.

Active layer thickness: is the dephts of the upper part of permafrost, which freezes and thawes every year (see Guglielmin's IPA-CALM protocol for details).

Soil Moisture Classes: provides rough estimations on soil moisture following five classes

Other observations: provide informations useful to describe particular environmental conditions and/or processes that may influence vegetation development

Formation Type: here all the formations observed in the site should be listed, for each formation providing information on their % coverage, number of observable strata and, when possible, the dominant species. When specimens are collected, indicate their identifier (collection number) behind their name in this form.

Stratification: the number of strata and their height in the vegetation.

Soil Moisture Classes
1 = dry: no detectable moisture;
2 = slightly moist;
3 = moist: soil sample is malleable, water content is close to field capacity, no free water;
4 = very moist;
5 = saturated: the whole porosity is water saturated or the layer is under the water table.

Dominance
D = Dominant
C = Companion

Strata
C = Cryptogams layer
H = Herb Layer: H1 = 0<h<5cm; H2 = 5<h<10cm; H3 = 10<h<20cm; H4 = 20<h<30cm; H5 = h > 30 cm
S = Shrub Layer S1 = 30cm<h<2m; S2 = 2m<h<5m

Site Description for active layer monitoring - additional data

Observer	Site identifier / number	Photos:
		Samples:
Site Code (CALM-IPA)		
Date (dd/mm/yy)		
Geomorphological characteristics	□ Pro-Glacial □ Nival □ Chionophilous □ Periglacial □ Fluvial □ Coastal □ Fell-Field	□ Slope □ Plateau □ Valley □ Landslide □ Scree slope □ Rock wall □ Other
CALM-IPA data: GEOMORPHOLOGICAL (landform) DESCRIPTION OF AREA CONTAINING SITE:		
CALM-IPA data: GEOMORPHOLOGICAL (surface) DESCRIPTION OF THE SITE FOR THE GRID AREA (i.e. very rough surface, gravely with rare big boulders)		
CALM-IPA data: SURFACE TEXTURE DESCRIPTION of the Monitoring Point (predominant texture, i.e., 'sandy-gravel', 'gravel', 'silty-sand', etc.):		
CALM-IPA data: VEGETATION CHARACTERISTICS FOR THE GRID (i.e. % coverage; dominant group)		
Substrate (rock lithology)	□ Siliceous rock □ Calcareous rock	Rock Type
Active layer thickness		
Soil Moisture Class	Class Number	
Other observations	□ Salt crust or efflorecences □ Desert pavement □ Bird or mammals products (faeces, carrion, feathers,) □ Soil Erosion	

CALM-IPA data: LIST OF CLIMATE ELEMENTS RECORDED WITHIN THE SITE (i.e every changes in height of the sensors and Mean, Maximum and Minimum values for each one have to be reported here)	
CALM-IPA data: CLOSEST CLIMATE STATION (AWS) (name, lat./long.); INCLUDE MAAT, Minimum and maximum air temperature, and mean annual snow depth (when available) and list of climatic parameters recorded and their time interval	

List of vegetation formations			
Formation Type	Coverage(%)	Stratification	Dominant Species
Cryptogamic Vegetation			
□ Algae and cyanobacteria			
□ Crustose lichens dominated			
□ Foliose and fruticose lichens (macrolichens) dominated			
□ Lichen - dominated, with bryophytes			
□ □ Bryophyte - dominated, with lichens			
□ Bryophyte vegetation			
Herbaceous Vegetation			
□ Cryptogam - dominated with herbaceous plants			
□ Open pioneer grasslands			
□ Closed grasslands			
□ Open herbfield			
□ Closed herbfield			
Shrub Vegetation			
□ Dwarf shrub dominated			

ANNEX 3. Vegetation analysis / community description

(advanced level)

At least all characteristics as listed on the general observation / collection form (annex 1) should be recorded for each quadrat. Other characteristics are dependent on the precise objective of the vegetation analysis. Below we have listed the information that was collected during the RiSCC 3 islands project. When vegetation studies are to be related to permafrost studies, the additional information required should be recorded on the appropriate form (annex 2).
Environmental variables (e.g. soil moisture, organic matter content) may be measured or estimated. See Bergstrom & Selkirk (2000) for a definition of ordinal scales for a number of environmental variables (see below for definitions).
In the case of communities with a varied vertical structure (e.g. tussock grassland in seal colonies), a distinction between an upper and a lower herb layer can be useful.
For each species in addition to the cover % notes can be made on sociability, vitality and phenology. Scales for this can be found in e.g. Shimwell (1971), Mueller-Dombois & Ellenberg, 1974), Westhoff & van der Maarel (1973). Given the fact that the extra effort involved in collecting this information is negligible, we suggest doing this in all surveys.
For studies in areas with a restricted set of species, it may be useful to have the species list printed on the form, with for each species a field for cover, sociability, phenology and vitality.
When specimens are collected from the plots: note the collection number next to the species in the species list, and write the collection number + plot number on the collection label.
It is a good idea to take a picture of each plot. Note the identification of the picture on the form, and include the plot identification in the picture.
Soil (unconsolidated substrate) depth, measured with e.g. a 1-m length metal rod

Moisture / availability of water, estimated on a 5-point ordinal scale:

1 = dry;
2 = damp (not dry, but no water can be squeezed out of soil or bryophytes by hand),
3 = moist (strong pressure required before water drips from soil or bryophytes),
4 = wet (water drips from soil or bryophytes with light pressure) ,
5 = waterlogged, aquatic (water drips freely from soil or moss samples taken in these habitats).

Exposure to wind, estimated on a 5-point ordinal scale:

1 = strongly sheltered (e.g. caves, crevices, ground-layer in dense tussock grassland);
2 = somewhat sheltered;
3 = moderately exposed, but not from all sides, somewhat sheltered from the direction of the predominant winds;
4 = exposed, between 3 and 5;
5 = strongly exposed to wind from all directions, usually at high altitudes.

Influx of sea salt / saltspray, estimated on a 5-point ordinal scale:

1 = no influence;
2 (very rarely exposed to saltspray) and
3 = moderate influence (coastal areas not exposed to prevailing winds, saltspray quantities low);
4 (regularly exposed to saltspray, but usually in relatively small quantities) can be used.
5 = strong influence (low-lying areas along the coast, exposed to the prevailing winds, with heavy saltspray);

Nutrient enrichment by animals, estimated on a 5-point ordinal scale:

1 = no influence;
2 = slight influence;
3 = moderate influence (e.g. near burrowing birds nests);
4 = strong influence (areas around bird colonies, seal colonies with not very high densities);
5 = very strong influence (e.g. in and very close to penguin and seal colonies; seal, wallows)

species abundance:

species abundance for plants can be expressed as cover %, or as number of individuals per unit area, but for a rough idea of the abundance of the species a simple ordinal abundance scale may be sufficient:

r = rare - only one or a few individuals
o = occasional - not many individuals, dispersed throughout the area, cover low
f = frequent - quite large number of individuals, but no large cover
a = abundant - many individuals or large cover
d = dominant or co-dominant- species reaching large cover

Cover is preferably estimated directly in % of total area. Alternatively the extended combined cover-abundance scale of Braun-Blanquet can be used (see e.g. Table II in Westhoff & van der Maarel, 1973).

Sociability (Braun Blanquet):

1 = growing solitary, singly
2 = forming small groups of a few individuals, or in small tufts
3 = forming small patches or cushions, or large tufts
4 = growing in extensive patches or broken mats
5 = growing in great crowds or extensive mats, completely covering the whole plot area; often large, almost pure stands

Phenology (for more detailed studies the scale given in the relevant chapter oif this manual should be used)

s = seedling
v (or no index) = vegetative
b = budding
fl = flowering
fr = fruiting
d = dead

Vitality (Braun Blanquet / Mueller-Dombois & Ellenberg):

°° = 1 = very feeble, and never fruiting
° = 2 = Feeble
no index = 3 = normal
• = 4 = exceptionally vigorous

RiSCC Vegetation analysis

Project		observer:
quadrat number:	date:	plot size:
location:	lat/long:	
habitat:		
vegetation:		
		size of community:
elevation:	slope:	exposure/shelter:
soiltype:	soildepth:	substratum stability:
		soilsample nr.:
groundwaterdepth:	moisture:	Soil water content:
pH:	conductivity:	
soil organic matter:		
salt:	animal excreta:	trampling:
surrounding vegetation and habitat:		

cover herblayer:	cover mosslayer:	cover lichens:
height herblayer:	height mosslayer:	height lichens:
cover aliens:	cover algae:	
cover rocks:	cover bare soil:	

species name	cover	soc.	phen.	vit.	species name	cover	soc.	phen.	vit.

Notes

ANNEX 4. Soil description (Intermediate Level)

Soil Description

Survey Number :	Edaphic / floristic parameters: see Basic form n°
Date (dd/mm/yy) :	
Location Name: ? Site method Site Number ? Transect method Transect Number	
Geographical coordinat Lat. Long.	Slope (°)
Author:	

Depth cm	Diagram	N° Hz	Colour Munsell	Moisture class	Org. Matter class	Coarse elements		Texture	Structure	Roots			Pedological traits		
						size class	%			size class	abundance	sanitary st.	nature	colour	cover %
		Litter													
0-															
10-															
20-															
30-															
40-															
50-															
60-															
70-															
80-															
90-															
100-															

End by choice:
truncated: to

Photos n°:

Samples:

water-table depth (cm):

Observations:

ANNEX 5. MP: Monitoring Points with Year-round Measurements (Advanced Level)

SITE NAME:

SITE CODE

MP ELEVATION (elevation a.s.l. meters):

MP SLOPE (°) AND ASPECT (°):

MP POSITION (grid number, see Figure):

DEPTHS OF THE SENSORS:

TYPE OF SENSORS AND ACCURANCY:

TYPE OF INSTALLATION (only if it is different from the protocol):

TIME INTERVAL (only if it is different from the protocol):

SURFACE TEXTURE CHARACTERITICS (i.e sand):

PRESENCE AND THICKNESS OF ORGANIC MAT LAYER (at the surface):

BRIEF DESCRIPTION OF THE LITHOLOGY (granulometric and lithological differences within the terrain until the monitored depth, indicating where ice occurs):

VEGETATION CHARACTERISTICS (cover in % and type of community):

LIST OF CLIMATE ELEMENTS RECORDED WITHIN THE SITE (Mean, Maximum and Minimum values for each one have to be reported here. Note down all changes in height etc. of the sensors, with the date these changes were made):

_____

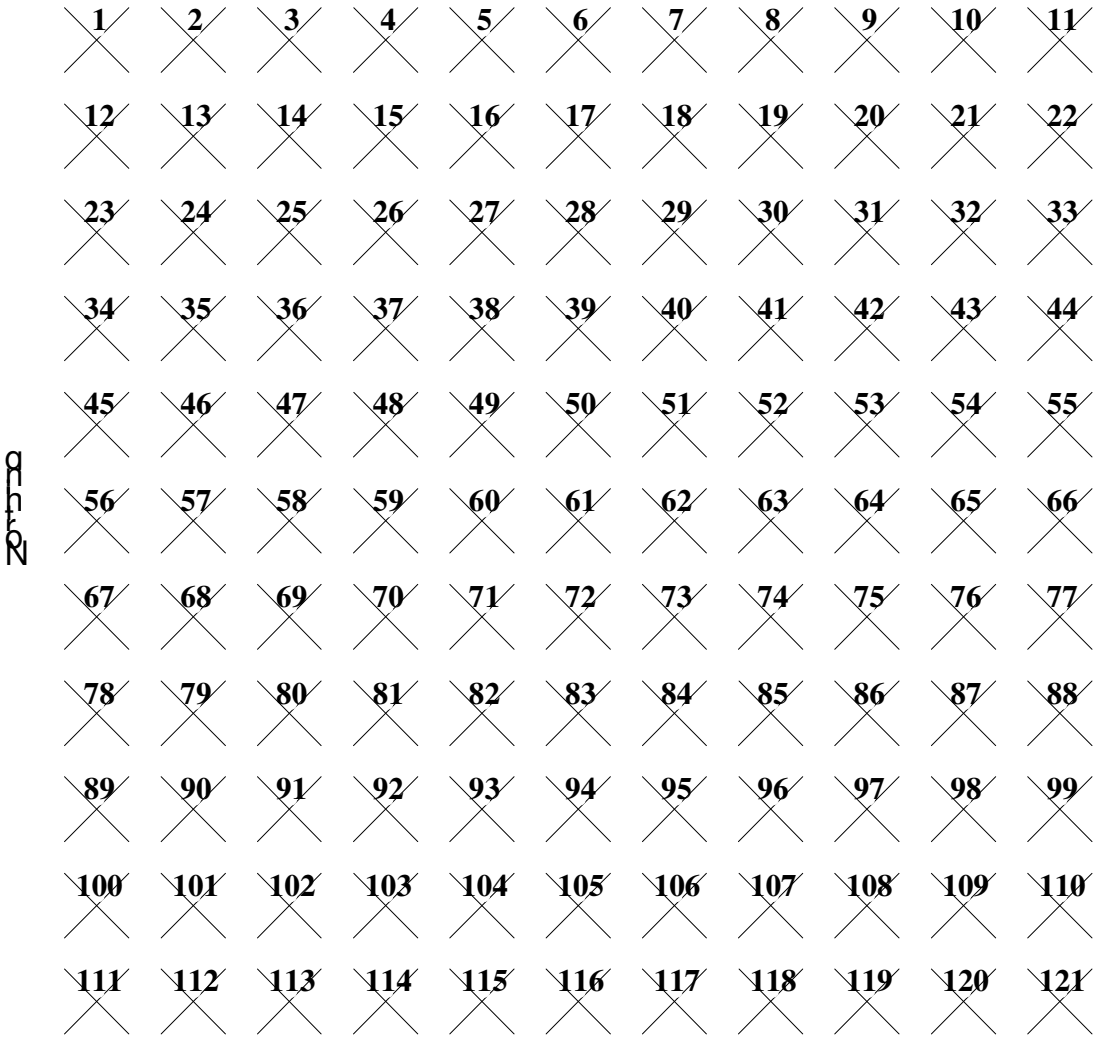
_____

ADDITIONAL COMMENTS:

ANNEX 6. GTPM: Grid Thermal Periodic Monitoring

(Intermediate Level)

SITE:_____ DATE:



Max: _____ Min: _____ Mean: _____

Std. Dev: _____ N of nodes with snow: _____

ANNEX 7. Phenology and Morphometry

(Basic Level)

Below is the form used in a studie of the phenology of *Acaena magellanica*, provided by Dana Bergstrom and Marc Lebouvier. This form can be simply adapted for other species. Also added is a form that is used in a study of the phenology and morphology of *Deschampsia antarctica*, provided by Ad Huiskes.

It is suggested in addition to providing the site information on the general site description form (annex 1), to add at least information on moisture, nutrient status, saltspray and nutrient enrichment. Other information may be useful as well, e.g. such as what is asked for in the top half of the vegetation analysis form (Annex 3).

Initial *Acaena magellanica*
Site
Date

Plant #		*A-1*	*A-2*	*A-3*	*A-4*	*A-5*	*A-6*	*A-7*	*A-8*	*A-9*	*A-10*	*A-11*	*A-12*	*A-13*	*A-14*	*A-15*	*A-16*	*A-17*	*A-18*	*A-19*	*A-20*	Mean
	Height mm																					
	Width mm																					
Distribution Classes	Isolated individuals																					
	Small clumps																					
	Med clumps																					
	large clumps																					
	Continuous cover																					
Vigour	poor																					
	average																					
	good																					
Phenology classes- *% of each class*	aborted (N)																					
	young buds																					
	style erect																					
	style dry / flacid, anthers fat or open																					
	anthers old / dry																					
	spike emerging																					
	spike well emerged > 2 mm																					
	fruit shedding																					
	fruit shedding previously collected																					
	Total without aborted																					
	comment																					

Phenological and morphological observations. species:

Phenological observations: (enter Y (Yes) or N (No))

Replicates	Snow-free?	Fully devlpd leaves?	Infl. developing	Infl. app. from sheath	Flower(s) open?	Anthers open?	Flowers fading?	Seeds ripe?
1								
2								
3								
4								
5								
6								
7								
8								
9								
10								

Observer:

Date:

Site identification:

Remarks:

Vegetative growth (5 shoots/genet)

Generative growth (5 inflorescences/genet)

Replicate genets	length (mm)					nr. of fully devlpd leaves					nr. Inflor./genet					nr. flowers/inflor.					nr. "seeds"/inflor.				
	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5
1																									
2																									
3																									
4																									
5																									
6																									
7																									
8																									
9																									
10																									

ANNEX 8. Sampling for biochemical studies (stress, markers) and genetics

(Advanced Level) - see next page for a guide for sample fixation and conservation

SAMPLING FOR BIOCHEMICAL AND GENETICAL STUDIES

10 replicates as a rule - minimum 5 required.
For all samples avoid unnecessary wounding when collecting.
Frozen samples must be kept frozen until analysis.

The following table is the form which should be filled up in any sampling for biochemical or genetical research.
This form should come in complement to the basic form describing site, vegetation etc.
Note that most details are under the form of comments to the title boxes and are aimed at helping the collector to write the appropriate information down, depending on the study.

Sample ID	Collection time	Sample description	Fresh Weight	Time before fixation	Storage conditions	After-fixation events (break in cooling, thaw…)

The next table should serve as a guidance for the most common biochemical and genetical studies.
Of course, in each study, a detailed sampling / fixation / conservation protocole should be provided by the scientist.

SAMPLE FIXATION/ CONSERVATION GUIDE

Type of analysis		Immediate fixing/ freezing required	Appropriate type of collection						
			Frozen in liquid N2	Frozen (-20°C)	Solvent	Oven dried	Microwave dried	Silica dried	Lyophilised
DNA damage		Yes	X						
DNA sequencing			X	X			X		
Photosynthetic pigment		Yes	X						X
UV-B absorbing pigments			X	X		X			X
polyols/ HPLC		Yes	X	X					X
sugars/ HPLC		Yes	X	X					X
amino acids/ HPLC		Yes	X	X					X
Polyamines	**Free**	Yes	X	X	Hydrochloric Acid 1N				X
	Conjugated	Yes	X	X	Methanol 100%				
NMR		Yes	X	(X)	Perchloric Acid				X

ANNEX 9. Description of aquatic non-marine systems for non-limnologists

Basic level: only requires a map, an ice-axe, a pencil and 30 min

Identifier:			Date/Time:
Observer:			
Location (coordinates)(1) :			
Altitude:			
Name of area and/or water body:			
Type:	Lake/stream/lagoon		
Size:	diameter		Estimated / measured
	perimeter		Estimated / measured
Depth:			Estimated / measured
Inflows:	Yes/No	How many?	
Outflows	Yes/No	How many?	
Ice coverage	permanent	Yes/No	
	% coverage (up to): 25, 50, 75, 100%		
	Ice thickness:		Estimated / measured
	ice transparency: high, moderate, none		
Water colour: clear, brown (organic), milky, others			
Zooplankton	Yes/No		
Lake Shores:	Vegetated (up to): 25%, 50%, 75%, 100%		
	cyanobacterial mats (2): Yes/No		
	green algae (3): Yes/No		
	mosses: Yes/No		
	vascular plants: Yes/No		
Catchment	Size (4): 1x size of waterbody, 10x, more		
	Vegetated (up to): 25%, 50%, 75%, 100%		
	Vegetation:moss / lichens / vascular plants		
	Geology (e.g.): volcanic, metamorphic, granitic, sandstone		
	Geomorphology : slope: flat, steep, cliffs		
	Geomorphology: weathering: none, some, extensive		
	Animal influence intensity: negligible / moderate / high / intense		
	Animal influence type: nesting birds / marine mammals		
	Snow and ice (up to): 25%, 50%, 75%, 100%		
	General wetness of the area (polar desert, wet polar area?)		
Other Observations:			

1- *give the GPS coordinates of at least one point at the water body or compass bearings to prominent landmarks*

2- *cyanobacterial mats can be detected by running a finger through the surface of sediments, the appearence would be a different colour than the sediments (brown, orange, blue-green)*

3- *green algae have the aspect of bright green colour filaments or sheets*

4- *size of catchment in reference to the waterbody*

Sketch of waterbody and ice coverage:

ANNEX 10. Fresh and brackish water Antarctic algae literature

Check-lists

Cavacini P., Fumanti B. (2001): A check-list of non marine microalgae of Continental Antarctica. SCAR 8th Biology Symposium, Amsterdam, 27 August – 1 September 2001 (Abstracts): 1.

Hakansson H., Jones V.J. (1994): The compiled freshwater diatom taxa list for the maritime Antarctic region of the South Shetland and South Orkney Islands. P.B Hamilton (Ed.), Proc. 4th Arctic-Antarctic Diatom Symposium. Canadian Museum of Nature, Ottawa, Canada.

Hirano, M. (1965): Freshwater algae in the Antarctic regions. In: J. Van Mieghem, P. Van Oye & J. Schell (Eds.), Biogeography and ecology in Antarctica. Monographiae Biologicae, 15: 127-193.

Prescott, G.W. (1979): A contribution to a bibliography of Antarctic and Subantarctic Algae. Bibl. Phycol., 45. J. Cramer, Vaduz. 121 pp.

Selected publications on Antarctic microalgae

The results of the investigations on Antarctic microalgae are scattered in many papers generally concern the microflora of more or less limited ice-free zones of the Continent. These papers are very useful for Antarctic specimen's comparison, especially if draws and photographs are added. Unfortunately they are relatively a few so the use of the pioneer's explorations literature, with old draws and descriptions at Light Microscope, until now represents the best instrument for comparison. The following references list only reports the most accurate Antarctic fresh- and brackish- waters algal floras and some papers on Antarctic algae of limnetic ecosystems (especially those with photos and draws) published in the period 1993-2001 (for older papers see Broady, 1992).

Alger A.S. (1997): Ecological processes in a cold desert ecosystem: abundance and species distribution of algal mats in glacial meltwater streams in Taylor Valley, Antarctica. Univ. Colorado. Institute of Arctic and Alpine Res., 51: 34-36.

Alger A.S., McKnight D.M., Spaulding S.A., Tate C.M., Shupe G.H., Welch K.A., Edwards R., Andrews E.D., House H.R. (1996): Ecological processes in a Cold Desert Ecosystem: the abundance and species distribution of algal mats in glacial meltwater streams in Taylor Valley, Antarctica. U. S. Geological Survey, Boulder, Co. 102 pp.

Alger A.S., Stoermer E.F., McKnight D.M. (1997): Factors influencing algal abundance and species distribution in meltwater streams in Taylor Valley, Antarctica. Phycologia, 36 (Suppl. 4): 1.

Broady P.A. (1982): Taxonomy and ecology of algae in a freshwater stream in Taylor Valley, Victoria Land, Antarctica. Arch. Hydrobiol. / Suppl. 63, 3, Algological Studies, 32: 331-349.

Carlson G.W.F. (1913): Süßwasseralgen aus der Antarktis, Südgeorgien und der Falkland Inseln. Wiss. ergebn. Schwedischen Südpolar Expedition 1901-1903, 4. Stockholm. 94 pp.

Cavacini P. (1999): Preliminary account of algae from two lakes of Tarn Flat (northern Victoria Land, Antarctica). Newsletter of the Italian Biol. Res. in Antarctica, 3: 35-39.

Ellis-Evans J.C., Laybourn-Parry J., Bayliss P.R., Perriss S.J. (1998): Physical, chemical and microbial community characteristics of lakes of the Larsemann Hills, Continental Antarctica. Arch. Hydrobiol., 141 (2): 209-230.

Fritsch F.E. (1912a): Freshwater algae of South Orkneys. Linn. Soc. Journ. Bot., 40: 293-338.

Fritsch F.E. (1912b): Freshwater algae. In: National Antarctic Expedition. Natural History. Br. Mus. Nat. Hist., 6. 54 pp.

Fritsch F.E. (1917): Freshwater algae. In: British Antarctic "Terra Nova" Exped. 1910. Br. Mus. Nat. Hist. Rep. Botany, 1: 1-16.

Fritsen C.H., Priscu J.C. (1998): Cyanobacterial assemblages in permanent ice covers on Antarctic lakes: distribution, growth rate, and temperature response of photosynthesis. J. Phycol., 34 (4): 587-597.

Fukushima H. (1997): Freshwater plankton in a pond at Cape Adare. Polar News, 32 (2): 15-19.

Fumanti B., Alfinito S., Cavacini P. (1995): Floristic studies on freshwater algae of Lake Gondwana, Northern Victoria Land (Antarctica). Hydrobiologia, 316: 81-90.

Fumanti B., Cavacini P., Alfinito S. (1997): Benthic algal mats of some lakes of Inexpressible Island (northern Victoria Land, Antarctica). Polar Biol., 17: 25-30.

Gain M.L. (1912): La flore algologique des regions antarctiques et subantarctiques. Deuxieme expedition Antarctique française commandee par J. Charchot (1908-1910). Sci. Nat. Doc. Sci. Paris. 202 pp.

Hirano M. (1979): Freshwater algae from Yukidori Zawa, near Syowa Station, Antarctica. Mem. Natl. Inst. Polar Res., 11: 1-25.

Hirano M. (1983): Freshwater Algae from Skarvsnes, near Syowa station, Antarctica. Mem. Nat. Inst. Polar Research, Ser E. Biological and Medical Science, 35: 1-31.

Izaguirre I. (1997): Summer phytoplankton populations of Lake Boeckella. Inst. Antarctico Argentino, 449: 13-14.

Izaguirre I., Pizarro H. (1998): Epilithic algae in a glacial stream at Hope Bay (Antarctica). Polar Biol., 19: 24-31.

James M.R., Pridmore R.D., Cummings V.J. (1995): Planktonic communities of melt ponds on the McMurdo Ice Shelf, Antarctica. Polar Biol., 15: 555-567.

Jones V.J. (1993): The use of diatoms in lake sediments to investigate environmental history in the maritime Antarctic: an example from Sombre Lake, Signy Island. In: R.B. Heywood (Ed.) University Research in Antarctica. British Antarctic Survey: 91-96.

Kawecka B., Olech M., Nowogrodzka-Zagorska A M., Wojtun B. (1998): Diatom communities in small water bodies at H. Arctowski polish Antarctic Station (King George Island, South Shetland Islands, Antarctica). Polar Biol., 19: 183-192.

Kellogg D.E., Stuiver M., Kellogg T.B., Denton G.H. (1980): Non-marine diatoms from Late Wisconsin perched deltas in Taylor Valley, Antarctica. Palaeogeography, Paleoclimatology and Palaeoecology 30:157-189.

Kloser H. (1998): Habitats and distribution patterns of benthic diatoms in Potter Cove (King George Island, Antarctica) and its vicinity. Ber. Polarforschung, 299: 95-105.

Komárek J. (1999): Diversity of Cyanoprokaryotes (Cyanobacteria) of King George Island, Maritime Antarctica - a survey. Algological Studies, 94: 181-193.

Komárek O., Komárek J. (1999): Diversity of freshwater and terrestrial habitats and their oxyphototroph microflora in the Arctowski Station region, South Shetland Islands. Pol. Polar Res., 20 (3): 259-282.

Laybourn-Parry J., Bayliss P., Ellis-Evans J.C. (1995): The dynamics of heterotrophic nanoflagellates and bacterioplankton in a large ultra-oligotrophic Antarctic-lakes. J. Plank. Res., 18: 495-511

Laybourn-Parry J., James M.R., McKnight D.M., Priscu J., Spaulding S.A., Shiel R. (1997): The microbial plankton of Lake Fryxell, southern Victoria Land, Antarctica during the summers of 1922 and 1994. Polar Biol., 17: 54-61.

Ling H.U., Seppelt R.D. (1998): Non-marine algae and cyanobacteria of the Windmill Islands region, Antarctica with descriptions of two new species. Algological Studies, 89: 49-62.

Martinez-Macchiavello J.C., Salas Aramburu W. (1994): Taxonomic and ecological catalogue of diatoms from Argentina and Antarctic insular lakes. Inst. Antarctico Argentino, Publ. 27: 1-113.

Massalski A., Mrozinska T., Olech M. (1999): Ultrastructure of selected *Cyanophyta/Cyanobacteria* from King George Island, Antarctica. Algological Studies, 94: 249-259.

Mataloni G., Tesolin G., Tell G. (1998): Charcterization of a small eutrophic Antarctic lake (Otero Lake, Cierva Point) on the basis of algal assemblages and water chemistry. Polar Biol., 19: 107-114.

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ANNEX 11. Protozoans literature

Useful literature for ciliate species identification

Both live observations and silver impregnations are often necessary for correct ciliate species identifications. However, with some taxonomic experience it is often possible to identify many species already *in vivo*.

Currently, there is no single monograph for the identification of freshwater or soil ciliates either in the Antarctic or elsewhere. As new species are continuously described, it is necessary to survey the literature. However, there are compilations of some systematic groups but some of these are in German. Several publications are already dated but still useful.

Terrestrial ciliates

Berger H. (1999): Monograph of the Oxytrichidae (Ciliophora, Hypotrichida). Kluwer, Dordrecht. 1079 pp.

Foissner W. (1993): Colpodea (Ciliophora). G. Fischer, Stuttgart. 798 pp.

Foissner W. (1996): Faunistics, taxonomy and ecology of moss and soil ciliates (Protozoa, Ciliophora) from Antarctica, with description of new species, including *Pleuroplitoides smithi* gen. n., sp. n. Acta Protozool., 35: 95-123.

Kahl A. (1926): Neue und wenig bekannte Formen der holotrichen und heterotrichen Ciliaten. Arch. Protistenkd., 55: 197-438.

Kahl A. (1927a): Neue und ergänzende Beobachtungen holotricher Ciliaten. I. Arch. Protistenkd., 60: 34-128.

Kahl A. (1927b): Neue und ergänzende Beobachtungen heterotricher Ciliaten. Arch. Protistenkd., 57: 121-203.

Kahl A. (1930a): Neue und ergänzende Beobachtungen holotricher Infusorien. II. Arch. Protistenkd., 70: 313-416.

Kahl A. (1930b): Urtiere oder Protozoa I: Wimpertiere oder Ciliata (Infusoria). 1. Allgemeiner Teil und Prostomata. Tierwelt Dtl., 18: 1-180.

Kahl A. (1931): Urtiere oder Protozoa I: Wimpertiere oder Ciliata (Infusoria). 2. Holotricha. Tierwelt Dtl., 21: 181-398.

Kahl A. (1932): Urtiere oder Protozoa I: Wimpertiere oder Ciliata (Infusoria). 3. Spirotricha. Tierwelt Dtl., 25: 399-650.

Kahl A. (1935): Urtiere oder Protozoa I: Wimpertiere oder Ciliata (Infusoria). 4. Peritricha und Chonotricha. Tierwelt Dtl., 30: 651-886.

Petz W., Foissner W. (1996): Morphology and morphogenesis of *Lamtostyla edaphoni* Berger and Foissner and *Onychodromopsis flexilis* Stokes, two hypotrichs (Protozoa: Ciliophora) from Antarctic soils. Acta Protozool., 35: 257-280.

Petz W., Foissner W. (1997): Morphology and infraciliature of some soil ciliates (Protozoa, Ciliophora) from continental Antarctica, with notes on the morphogenesis of *Sterkiella histriomuscorum*. Polar Rec., 33: 307-326.

Berger H. (1999): Monograph of the Oxytrichidae (Ciliophora, Hypotrichida). Kluwer, Dordrecht. 1079 pp.

Foissner W. (1993): Colpodea (Ciliophora). G. Fischer, Stuttgart. 798 pp.

Foissner W., Berger H. (1996): A user-friendly guide to the ciliates (Protozoa, Ciliophora) commonly used by hydrobiologists as bioindicators in rivers, lakes, and waste waters, with notes on their ecology. Freshwater Biol., 35: 375-482.

Foissner W., Berger H., Kohmann F. (1992): Taxonomische und ökologische Revision der Ciliaten des Saprobiensystems. II. Peritrichia, Heterotrichia, Odontostomatida. Informationsber. Bayer. Landesamtes Wasserwirtsch., 5/92: 1-502.

Foissner W., Berger H., Kohmann F. (1994): Taxonomische und ökologische Revision der Ciliaten des Saprobiensystems. III: Hymenostomata, Prostomatida, Nassulida. Informationsber. Bayer. Landesamtes Wasserwirtsch., 1/94: 1-548.

Foissner W., Berger H., Schaumburg J. (1999): Identification and ecology of limnetic plankton ciliates. Informationsber. Bayer. Landesamtes Wasserwirtsch., 3/99: 1-793.

Foissner W., Berger H., Blatterer H., Kohmann F. (1995): Taxonomische und ökologische Revision der Ciliaten des Saprobiensystems. IV: Gymnostomatea, *Loxodes*, Suctoria. Informationsber. Bayer. Landesamtes Wasserwirtsch., 1/95: 1-540.

Foissner W., Blatterer H., Berger H., Kohmann F. (1991): Taxonomische und ökologische Revision der Ciliaten des Saprobiensystems. I: Cyrtophorida, Oligotrichida, Hypotrichia, Colpodea. Informationsber. Bayer. Landesamtes Wasserwirtsch., 1/91: 1-478.

Kahl A. (1926): Neue und wenig bekannte Formen der holotrichen und heterotrichen Ciliaten. Arch. Protistenkd., 55: 197-438.

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Kahl A. (1934): Suctoria. Tierwelt Nord- und Ostsee, 26 (Teil II, c5): 184-226.

Kahl A. (1935): Urtiere oder Protozoa I: Wimpertiere oder Ciliata (Infusoria). 4. Peritricha und Chonotricha. Tierwelt Dtl., 30: 651-886.

Matthes D., Guhl W., Haider G. (1988): Suctoria und Urceolariidae (Peritricha). G. Fischer, Stuttgart. 309 pp.

Thompson J.C., Jr. (1972): Ciliated protozoa of the Antarctic Peninsula. Antarct. Res. Ser., 20: 261-288.

Thompson J.C., Jr., Croom J.M. (1978): Systematics and ecology of ciliated protozoa from King George Island, South Shetland Islands. Antarct. Res. Ser., 27: 41-67.

Useful literature for testate amoebae species identification

Testate amoebae are usually identified by test morphology only. Currently, there is no single monograph for the identification of benthic freshwater, moss or soil testate amoebae either in the Antarctic or elsewhere. Listed below are thus compilations of systematic groups and some more comprehensive works.

Terrestrial testate amoebae

Bonnet L., Thomas R. (1960): Thécamoebiens du sol. Faune terrestre et d' eau douce des Pyrénées-Orientales, 5: 1-103.

Decloitre L. (1960): Thécamoebiens de la 8e expédition Antarctique Francaise. Bull. Mus. natn. Hist. Nat., 32: 242-251.

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Decloitre L. (1982): Compléments aux publications précédentes mise à jour 31. XII. 1981 des genres *Arcella*, *Centropyxis*, *Cyclopyxis*, *Euglypha*, *Nebela* et *Trinema*. Arch. Protistenkd., 126: 393-407.

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Smith H.G. (1978): The distribution and ecology of terrestrial protozoa of sub-Antarctic and maritime Antarctic islands. Br. Antarct. Surv. Sci. Rep., 95: 1-104.

Limnetic testate amoebae

Decloitre L. (1964): Thecamoebiens de la XII^{e} expedition Antarctique Francaise. Publs Expéd. polair. fr., 259: 1-47.

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Penard E. (1890): Études sur les rhizopodes d' eau douce. Mem. Soc. phys. hist. nat. Genève, 31: 1-228.

Penard E. (1902): Faune rhizopodique du bassin du Léman. H. Kündig, Genève. 714 pp.

Penard E. (1911): Sarcodina. Rhizopodes d' eau douce. Brit. Antarct. Exped. 1907-9, 1: 203-262.

Penard E. (1913): Rhizopodes d' eau douce. Deux. Expéd. franc., 1: 1-16.

Useful literature for flagellate species identification

No modern monographs are available for the identification of free-living heterotrophic flagellates from soil or freshwater. Further literature on selected groups can be found in, e.g., Lee et al. (2002).

Huber-Pestalozzi G. (1955): Das Phytoplankton des Süßwassers. Systematik und Biologie. 4. Teil Euglenophyceen. Binnengewässer, 16: 1-606.

Lee J.J., Leedale G.F., Bradbury P. (2002): The illustrated guide to the protozoa. Society of Protozoologists, Lawrence (year 2000). 1432 pp.

Pascher A., Lemmermann E. (1913): Flagellatae II. Chrysomonadinae, Cryptomonadinae, Eugleninae, Chloromonadinae und gefärbte Flagellaten unsicherer Stellung. Süsswass.-Flora Dtl. Öst. Schweiz, 2: 1-192.

Pascher A., Lemmermann E. (1914): Flagellatae I. Allgemeiner Teil, Pantostomatinae, Protomastiginae, Distomatinae. Süsswass.-Flora Dtl. Öst. Schweiz, 1: 1-138.

Patterson D.J., Larsen J., Eds. (1991): The biology of free-living heterotrophic flagellates. Clarendon Press, Oxford.

Zhukov B.F. (1993): Atlas of freshwater heterotrophic flagellates (biology, ecology, taxonomy). Russian Academy of Sciences, Institute of Biology of Inland Waters, Borok (in Russian).